TREATMENT OF FERTILIZER INDUSTRY WASTE: A STUDY ON DENITRIFICATION

A Thesis submitted
In partial fulfilment of the requirements
for the Degree of

MASTER OF TECHNOLOGY

by
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to the
Department of Civil Engineering
Indian Institute of Technology Kanpur

November 1969

CERTIFICATE

This is to certify that the present work has been done under my supervision and the work has not been submitted elsewhere for a degree.

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ACKNOWLEDGEMENTS

I take this opportunity to express my sincere gratitude to Dr. Rashid Hayat Siddiqi whose guidance and help enabled the studies to be undertaken and to complete successfully.

Profound thanks are herein expressed to Dr. G.D. Agrawal and Dr. A.V.S. Prabhakara Rao for their useful advice from time to time.

Sincere thanks to all my colleagues who helped me in this work.

TABLE OF CONTENTS

LIST OF TABLES

1. INTRODUCTION

Primary purpose of waste-water treatment is to prevent degradation of receiving waters. A modern plant including secondary treatment is specifically designed to control the quantity of organic carbon in its effluent. Other components such as nitrogen and phosphorous are only slightly affected. These pollutants do not always cause deterioration in receiving water quality. However when discharged for a long time or in excess, such as waste from a fertilizer industry, result in stimulation of biological activity in the receiving water causing concern to sanitary engineer (1) (2). The problem of disposal of fertilizer waste is assuming wide interest in our country because of increasing establishment of fertilizer factories. As the tropical climate of India is ideally suited for aquatic plant growth, particularly algae, discharge of these wastes containing large quantities of nitrogen into natural water is becoming an ever increasing problem. Another matter which should be evaluatedin considering any individual case of treatment of waste prior to admitting it to a stream or other body of water is balancing the benefits from protection water quality against its cost to the waste production.

These are three well recognised ills resulting from excessive plant growths in a water body or entrophication. These are (a) Algal toxicity (b) Asthetic deterioration of water quality resulting in costly water treatment operation and (c) Build up of Biochemical Oxygen Demand of water (2).

The build up of oxygen demand may be due to a large concentration of algal cells which require oxygen for respiration during dark hours of the day. Thus large concentration of algal cells produces supersaturation in the day light hours and oxygen depletion at night. These are extremes of environment and few lives tolerate such quick changes to extremes, either moving from the area if possible or being killed. This situation, however, is not as bad as that resulting from the death of algal crop. An algal crop may die from a toxic level of metabolites reached by its own activity or due to its sedimentation from the photic zone at high concentrations. A dead crops behaves like decomposible organic matter and therefore exerts oxygen demand. Literature concerning fishries contains many instance of fish kills due to such condition (3).

In the nitrogenous fertilizer industry a certain amount of nitrogen invariably finds its way into the factory effluent. Such effluent contain free ammonia, ammonium salts and some times also nitrates, all of which are undesirable in river water beyond a certain conentration. The former two are harmful for normal fish population while the latter in high concentration makes water nonpotable. Nitrate content in drinking water when exceed 20 mg/l of nitrogen interferes with the transport of oxygen from the lungs to body tissue and cause a condition,in infants known as methomoglobinemia (4). Therefore with the introduction of fertilizer industry effluent the

waste treatment problem involving high removal of nitrogen has suddenly mushroomed into one of major importance and of different type. This requires exparision of present plants or modification to provide a different type of treatment. As conventional treatment processes can not eleminate high concentration of nitrogen.

2. LITERATURE REVIEW

2.1 History of Indian Fertiliser Industry

The history of Indian fertiliser industry begins with the opening of ammonia plant at Belagula in Mysore. The first public sector fertiliser industry in India was started at Sindari in Bihar in 1944. The industry is expanding since then. Presently in India, eight plants are manufacturing nitorgenous fertilizers, six are under construction and are expected to go into operation by 1971. And other five are being proposed. The capacities of these plants range from 20,000 to 200,000 tonnes of nitrogen per annum. The consumption targets and distribution of various fertiliser plant are shown in figures 1 and 2 (5,6). In India presently the emphasis has been mostly given to the production of ammonical or nitrogenous fertilisers (7). Evidently the treatment to remove nitrogen is of increasing concern for water pollution control agencies and public.

2.2 Waste Characteristics

Detail study regarding the waste characteristic of fertiliser industry has not yet been carried out in India. However hardly one can have any doubt about its being enormously rich in nitrogenous compounds. Table 1 and 2 show the characteristics observed in the samples collected from two effluent streams of Sindari Fertiliser Plant P.O. Sindri, Dhanbad, Bihar (8) and the characteristic of waste-water expected from the urea Fertiliser Plant, Panki, Kanpur, Uttar Pradesh (9).

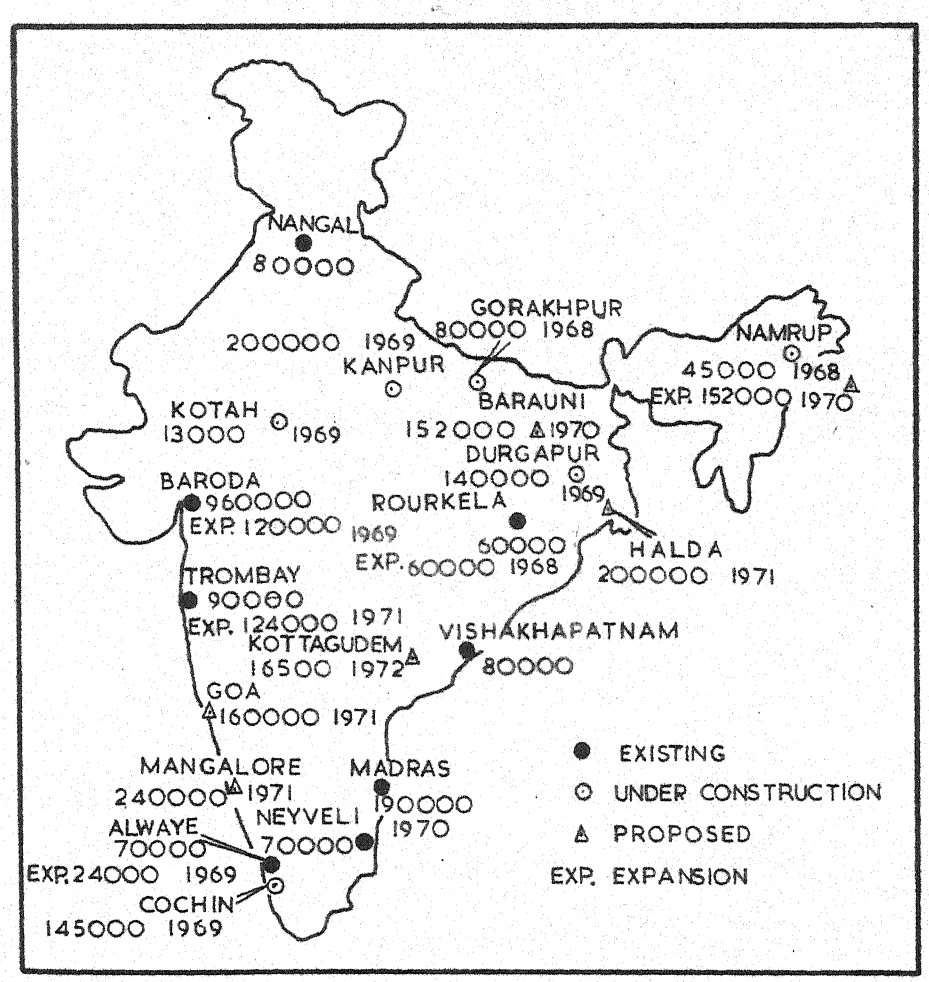


FIG. 1. MAJOR NITROGENOUS FERTILIZER PLANTS, AFTER S. LATEEF (5)

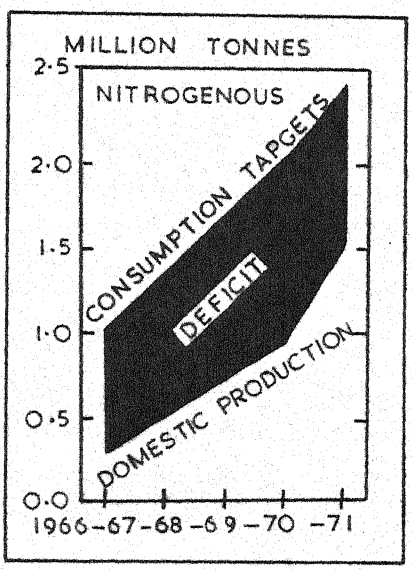


FIG. 2. CONSUMPTION TARGETS AND LIKELY AVAILABILITY OF FERTILIZERS, AFTER S. LATEEF (5)

TABLE 1

CHARACTERSTIC OF WASTEWATER AT SINDRI FERTILIZER UNIT, SINDRI

Description	Period	No. of Reading	Constituents	Analysis in ppm Max.	Min.	Ave.
Stream I	May to June, 1965	15	Total Phenol	8.0	0.2	1.6
			Cyanide as CN	5.8	0.05	1.5
			Amm. Nitrogen	238	7.0	88
			Suspended Solids	7190	960	3369
			Oil	24	1.0	8.2
			Flow in mgd	3.2	1.2	2.1
Stream II	May to June, 1965	15	Phenol	14.0	0.1	0.7
			Cyanide	3.2	0.4	1.2
			Amm. Nitrogen	939	342	313
			Suspended Solids	790	97	398
			Oil	29	2.0	11.5
			Flow in mgd	9.8	6.2	7.8

TABLE 2

ESTIMATED CHARACTERISTICS OF UREA PLANT, KANPUR

Item	kg/hr	ppm
Volume 107 M^3/hr		
Temperature 45°C		
Urea	114.7	1065
NH_3	149.3	1410
OO_2	200.5	1860
Other dissolved solids	136.4	1280
Total dissolved solids	251.1	2345
Suspended solids	-	-
Total solids	251.1	2345
Ammoniacal Nitrogen		1160

2.3 Nitrogen Removal Methods

With the exception of carbon and oxygen the nitrogen is the most prevelent element in algae. Therefore there is a natural inclination to regard it as the most logical point of attack to prevent turning over of an oligotrophic labes to eutrotophic one. Conventional treatment methods, highly effective in removing 80 to 95% BOD and suspended solids, do not normally remove enough of the nitrogenous pollutants. This requires expansion of present plants or modification to provide a different type of treatment. Following are the methods any one of which can be used for nitrogen removal

(a) Ion Exchange

(b) Oxidation Pond

(c) Ammonia stripping and

(d) Nitrification - Denitrification

2.3.1 Nitrification - Denitrification

Nitrogen can be removed from waste water by the progressive biological oxidation of nitrogen compounds to nitrites and nitrates followed by conversion to nitrogen gas. This two stage process terned nitrification - denitrification involues aerobic and anaerobic biological stages. Nitrification is typically achieved in an activated sludge tank by extending the normal areation time and by employing lower ratio of BOD to mix liquor suspended solids (10) than in conventional design. Denitrification results from facultative bacteria utilizing oxygen from the nitrates or nitrites. Since the nitrified effluent is deficient in carbon, this state of treatment requires the addition of an organic supplement (11)(12)(13) as hydrogen donating substrate. The denitrification step may be achieved in a separate tank with mechanical mixing and by developing a population of denitrifying organisms.

The fact that the process of nitrification - denitrification have been quite commonly observed in wastewater treatment plants encourages the use of this process for nitrogen removal. One of the early studies of nitrification and denitrification was by Sawer and Bradney (14) while they were investigating a rising sludge problem. Considerable pilot plant work

has been done in efforts to utilize the denitrification process for the removal of nitrogen. The first pilot plant work reported was that by Christianson et al (13) in describing experiment to reduce nitrate concentration from an industrial waste process. They tried chemical methods without success and ultimately used biological means of denitrification. They developed an anaerobic activated sludge using the nitrates in the waste as a soul source of oxygen and consequently nitrogen was lost by denitrification. The sludge they develope was gramular and of a low sludge density index. They used methanol as carbon source.

Wuhrmann (15) reported the loss of nitrogen by denitrification. Wuhrmann (16) in his continuous flow test reported that a domestic waste was treated in a conventional activated sludge arcation tank followed by an anaerobic denitrification unit and a final settling tank. He provided areation time of 1.7 to 2.2 hr. and provided sufficient mixed liquor solids to 0.33. A highly nitrified mixure was obtained and stirred anaerobically for 2.2 to 2.8 hours to obtain denitrification. He did not indicate the addition of any raw waste to the denitrification unit. Influent nitrogen concentration were 20 to 25 mg/l and effluent concentrations were 3 to 4 mg/l.

Several flow diagrams which have been used for denitrification process are presented in figure. 3.

The flow pattern shown in diagram A was used by Wuhrmann (17) in removing 60 to 80% of the incoming nitrogen.

Basically it is the conventional activated sludge process with a mechanically mixed denitrification tank place between the areation and the final settling tanks.

Diagram B involues a denitrification process entirely seperated from nitrification. In work by Johnson and Schroepfer (11) approximately two third of flow was tfeated for BOD removal in the conventional activated sludge plant and remaining one third was stabilized in unit utilizing nitrate-nitrogen.

Diagram C involue a semiaerobic activated sludge process utilizing denitrification for the elemination of nitrogen from waste water as used by Ludgac and Ettinger (18). The conventional areation tank was devided into two compartments one areated and one stirred mechanically. A baffle seperating the two compartments permitted recirculation of sludge from the areator into the denitrification unit through a slot at the bottom, and back to the areator over a weir at the top. Nitrogen removal varied from 50-75 percent under a range of operating conditions.

Haltrich (19) used the flow pattern shown in diagram D for the removal of nitrate from an industrial waste. He placed denitrification unit prior to the areation unit and obtained complete denitrification.

2.4 Process of Denitrification

The tern denitrification is sometimes confused by nitrate reduction. Any process in which an organism takes nitrate or nitrite from the surrounding medium, reduces this

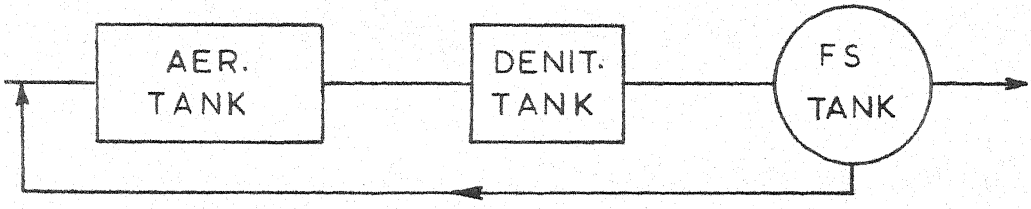


DIAGRAM A

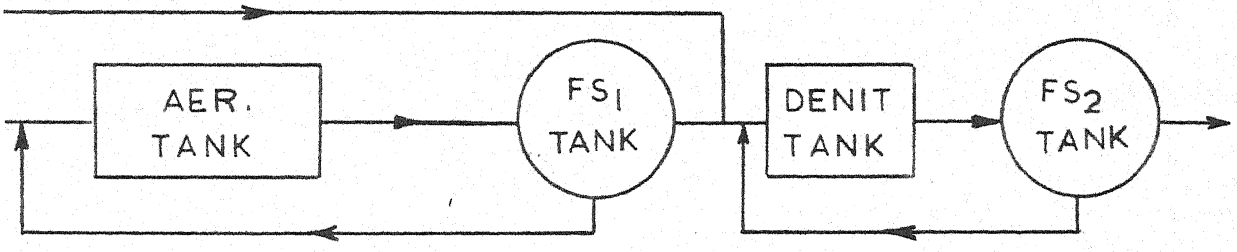


DIAGRAM B

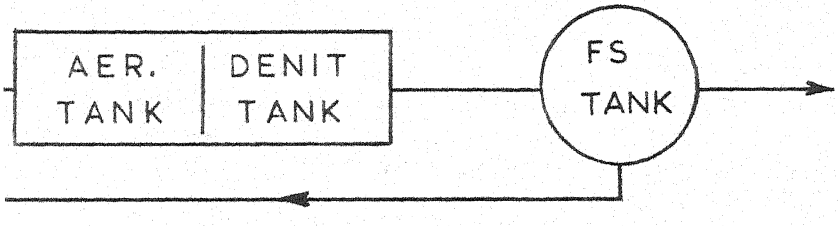


DIAGRAM C

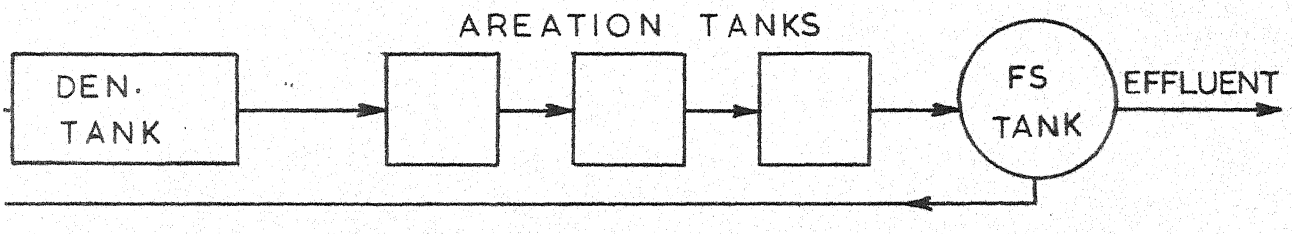


DIAGRAM D

FIG.3. FLOW DIAGRAMS

compound and transform it into cell protein may be termed nitrate reduction. They are two types; (a) Assimilatory nitrate reduction; In which nitrate is reduced only for the building up of cell protein. (b) Dissimilatory nitrate reduction: In which nitrate is used as hydrogen acceptors for energy oxidative reaction of micro-organism instead of oxygen. The ultimate products may be N_2 or N_{2O} gas or ammonia.

When the ultimate product are gaseous, N_2 or N_{2O}, the the reaction is termed as denitrification.

2.4.1 Denitrification Reaction

The pathway for denitrification reaction has not been fully established so far. Different people have proposed different mechanisms.

Gayson and Dupetit (20) proposed for the denitrification reaction overall equations, given below, which recognize the conversion of nitrate to nitrogen gas and carbohydrate to carbon dioxide and water but do not specify intermediate other than nitrite

$$2\ KNO_3 + C = 2\ KNO_2 + CO_2$$

$$4\ KNO_2 + 3C = 2\ N_2 + 2\ K_2CO_3 + CO_2$$

Beijerinck and Minckman (21) proposed another scheme in which nitrous oxide was included as an intermediate

$$2\ KNO_3 + 2C = N_{2O} + K_2CO_3 + CO_2$$

$$2\ KNO_2 + C = N_{2O} + K_2CO_3$$

$$2\ N_2O + C = 2N_2 + CO_2$$

Kluyver and Donker (22) emphasized that the reaction probably took place in two steps given below and included hyponitrite as an intermediate.

$$KNO_3 + 2H = KNO_2 + H_2O$$

$$2\,KNO_2 + 4H = K_2N_2O_2 + 2H_2O$$

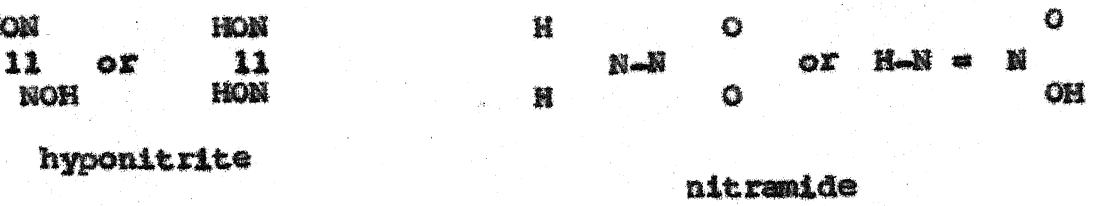
Potassium hyponitrite (unstable)

$$K_2N_2O_2 = N_2O + H_2O$$

Nitrous oxide thus evolved may further reduce to N_2 gas as follows.

$$N_2O + 2H = N_2 + H_2O$$

Allen and Najjar (23) proposed nitramide instead of hyponitrate which has the same empirical formula as hyponitrite but has different structure.



hyponitrite

nitramide

2.4.2 Process Control

Like all biological systems control of environmental condition of process denitrification is essential. The condition which are known to affect microbial activity are temperature pH, oxygen and the source of energy. The effect of these on denitrification process is summarized in the following sections.

2.4.2.1 Temperature

Much is not known about the effect of temperature on denitrifying bacteria. Denitrifiers do not appear to be quite as temperature dependent as the nitrifiers (24).

2.4.2.2 Oxygen Concentration

The reduction of nitrate and nitrite is essentially the use of bound oxygen as hydrogen acceptor. The denitrifiers, Therefore, requires that little or no dissolved oxygen be present for effecient operation (25). With the increase of free oxygen inhibition of denitrification reaction be expected. Deherain and Maquenne (26) indicated that denitrification took place only in the absence of oxygen. There are numerous conflicting reports on the influence of oxygen. Most workers agree that oxygen does inhibit the reduction of nitrate and the dis-agreement is largely on quantitative grounds. The inhibitory effect of oxygen on E Coli , using the reduction of nitrite, has shown in the table 3 (27) .

TABLE 3

EFFECT OF OXYGEN ON NITRATE REDUCTION BY E. Coli

Oxygen in gas %	Inhibition of nitrate reduction %
0	0
0.4	21
1.1	61
3.8	93
21	94
99	96

Sacks and Barker (28) in their studies with Ps denitrificans showed that 2.5% oxygen in the gas gave 45% inhibition while air (21%) gave 73% inhibition. Comparison of above with data of table 3 shows that compare to E. Coli denitrification by Ps denitrificans is not so readily suppressed by oxygen.

Nitrate reduction by Aerobacter almost stops when an anaerobically grown culture is areated (29). Nitrate reduction by the spore forming Denitrobac Licheniforms is inhibited only by vigorous areation (30). There are, however, exceptions to the observation that oxygen inhibts the reaction. Meiklejohn (31) studied two organisons of the genus preudomonas which she claimed denitrified aerobically, and similar observation are reported by Korsavokova (32).

Mechsner and Wuhrmann (33) has shown considerable variation in the ability of a number of strains of bacteria to cause denitrification at high concentration of dissolved oxygen.

2.4.2.3 Hydrogen Ion Concentration

The optimum pH for denitrification varies with the organism concerned. Karlsen (34) observed that Pseudomonas aeruginosa denitrify at hydrogen concentration ranging from pH 5.8 to 9.2 with an optimum value between pH 7.0 and 8.2. Wijler and Delwrche (35) in their studies with mix flora reported that at different hydrogen ion concentration the gaseous product evolued were different. The production evolued were different. The production of N_{2O} was favoured

at a neutral or alkaline pH. He observed that above pH 7.3 the N_2O gas evolved was readsorbed and further denitrify to nitrogen gas. Between pH 6 and 7 small evolution of N_3O gas was and at pH 5 the evolution of N_3O was 20% more than total nitrogen. Nitricoxide was also readsorbed and further denitrified, but not as rapidly as N_2O. Wuhrmann and Mechsner (36) in their studies with Spirillum virginianum have demonstrated that oxygen acts as a inhibitor for nitrite respiration at pH values above 5.8. With decreasing pH the detrimental effect of oxygen desappears.

2.4.2.4 Source of Energy and Carbon

Denitrifiers require are organic substrate to serve as a source of energy and carbon for building up protoplasm. The effluent from a nitrification tank is deficient in easily available dissolved compound which could be used as a respiration substrate by the sludge organisms. This stage of treatment therefore requires addition of an organic supplement (11) (12)(13).

Wuhrmann (36) in (37) his recent study has shown that the endogenous reserve materials in bactria are amply sufficient to maintain the respiration of the activated sludge untill all available nitrate or nitrite ions are reduced. He further noticed that this period of anaerobiosis does not have any harmful effect on the activity of activated sludge. He concluded that it is not necessary to add any exogenous source of hydrogen donor to the mixed liquor. Where as Jhonson and Schroepfer (11) observed that the

removal of nitrogen by denitrification is practically impossible without the addition of raw waste as a carbon source or electron donor. In the absence of organic substrate the rate is very slow. He showed that a ratio of oxygen resources to $\frac{5}{}$ day BOD in the raw waste of approximately 0.8 resulted in complete denitrification.

2.4.3 Organism Capable of Denitrification

Large number of species of wide variety of organisms are capable of denitrifying. These can be classified facultative in the sense that they are able to substitute nitrate or nitrite for oxygen as hydrogen acceptor. The denitrifiers studied by Gayon and Dupetit (20), Winogradsky (38), Buni and Stutzer (39) were all non-spore forming organisms of the genera Pseudomonas, Micrococcus and Spirillum. Lloyd (40) cites more than forty organisms which are capable of denitrifying. Beijerinck and Minckmann (21) observed denitrification by aerobic spore forming rocls. Other workers reported upon on denitrification by various "bacilli" most of them probably Pseudomonads. Waksman (41) isolated two organisms namely Ps. Pyocyanea producing N_2 and N_2O, the other Ps Stutzeri forming mainly N_2.

A species of Achromobacter described by Youatt (42) is of interest. It reduces nitrite to nitrogen gas but is incapable of reducing nitrate.

Some denitrifier are autotrophic in nature. Beijerinck (43) demonstrated denitrification with the oxidation of sulfur by Thiobacillus denitrificans and Thiobacillus thioparus . Kluyver and Verhoeuen (22) showed that Micrococcus denitrificans oxidized hydrogen at the expense of nitrate.

3 SCOPE OF STUDY AND OBJECTIVE

Johnson and Schroepfer (11) Whurmann (37), Haltrich (19) and others showed high potential for adopting biological nitrification-denitrification as a means of removing nitrogen from the waste. Whereas the study of Johnson and Schroepfer (11) was confined to the removal of nitrogen concentration (30 mg/l of nitrogen) slightly richer than present in domestic waste, Wuhrmann reported a nitrification-denitrification system suitable for removal of nitrogen upto concentration of 300 mg/l of nitrogen and Haltrich (19) carried out successful denitrification upto 400 mg/l of nitrate-nitrogen concentration by placing the denitrification tank before the activated sludge. However the kinetic aspects of the denitrification process for high concentration of nitrite and nitrate are still to be evaluated. Such work would be useful in designing and operation of treatment systems for wastes containing high concentration of various forms of inorganic nitrogen.

The objective of the present investigation included the following two aspects:

(1) Growth yield and rate of endogenous respiration of micro-organism in a denitrification system.

(2) To verify the kinetic expression currently used for describing growth of micro-organism in a single limiting substrate system for a denitrification system having two limiting substrates i.e. a source of energy and carbon (organic matter) and an electron accepton (nitrite).

4. EXPERIMENTAL DESIGN AND METHODS

4.1 Theoretical Background

4.1.1 Growth Yield and Endogenous Respiration Rate

Monod (44) proposed that for a given organism and given essential nutrient under similar condition the wight of bacteria produced per weight of nutrient utilized is remarkably constant. This has been since confirmed by many other microbiologist using various organisms and substrates. The relationship may be expressed as

$$Y = \frac{\text{Weight of organism formed}}{\text{Weight of essential nutrient utilized}} \quad .. \quad .. \quad (1)$$

where Y is the yield constant and a dimensionless number.

Moser (45) among others has employed the rate concept by expressing this as a differential equation.

$$d x = - Y \; d s \quad .. \quad .. \quad 2$$

where x is micro-organism concentration and s is substrate concentration.

Endogenous respiration rate is defined as the rate of oxygen utilization by a cell for oxidation of its own protoplasm. This effects the net yield of micro-organism. Its effect can be included by writting equation 2 as follows:

$$d x = - Y \; d s - K_e \; X_{av} \quad .. \quad .. \quad 3$$

where K_e is the endogenous respiration rate constant and X_{av} represents average concentrations of the micro-organism.

The above equation can be rewritten as

$$\frac{dx}{X_{av}} = Y \; \frac{ds}{X_{av}} - K_e \quad .. \quad .. \quad 4$$

Evidently a plot of $\frac{dx}{X_{av}}$ and $\frac{ds}{X_{av}}$ will result in a straight line. The values of slope and intercept with Y axis of this line will be equal to Y and K_e respectively.

In the present study the denitrification system consists of two substrates namely electron acceptor and donor (nitrite and peptone respectively). Therefore each growth yield and endogenous respiration rate constants will assume two different values. Equation 4 for the two case can be written ass

$$\frac{dx}{X_{av}} = - Y_1 \frac{ds}{X_{av}} - K_{e_1} \quad .. \quad .. \quad 5$$

and

$$\frac{dx}{X_{av}} = - Y_2 \frac{ds}{X_{av}} - K_{e_2} \quad .. \quad .. \quad 6$$

where subscript 1 and 2 denote constants for electron acceptor and donor respectively.

4.1.2 Kinetics of Substrate Removal

A growth rate equation for a system can be expressed by differential equation:

$$\frac{dx}{dt} = \mu x \quad .. \quad .. \quad 7$$

where μ is the specific growth rate constant and has a dimension of time inverse.

Monod (46) was the first to note a simple emperical relationship between the specific growth rate constant and the concentration of an essential nutrient. He described the relationship with a hyperbolic function similar to the Michaclies Mention equation used for describing euzyme substrate

$$\mu = \mu_{max} \frac{S}{K_s+S} \quad .. \quad .. \quad 8$$

where μ_{max} is the maximum value of growth rate constant and K_s is the saturation constant also known as Michaelies Menton constant.

It is seen that μ_{max} is the maximum value of specific growth rate at infinite substrate concentration ($S \gg K_s$) and has a dimension of time inverse. The saturation constant can be stated mathematically as follow:

$$K_s = (S) \quad \text{when} \quad \mu = \frac{\mu_{max}}{2}$$

i.e. it equals the concentration of substrate, at which specific growth rate is half the maximum growth rate.

For a denitrification system in which two substrates are rate limiting, the growth rate can be expressed as

$$\frac{dx}{dt} = \mu x \quad .. \quad .. \quad 9$$

substituting the values of and according to equation 8,

$$\frac{dx}{dt} = \mu_{max_1} \frac{S_1}{K_{s1}+S_1} \quad \mu_{max_2} \frac{S_2}{K_{s2}+S_2} \quad x \quad .. \quad .. \quad 10$$

The above equation integrates to

$$t = -\frac{M}{B} \ln \frac{A+B\,S_1}{A+B\,S_1O} + \frac{L}{E} \ln \frac{F-E\,S_1}{F-E\,S_1O} - N \ln \frac{S_1}{S_1O} \quad .. \quad .. \quad 11$$

where S_1O and S_1 is the nitrite concentration initially and at any time t, and A,B,E,F,L,M and N are the constants expressed as below.

$$A = S_2O - \frac{Y_1}{Y_2} S_1O \quad .. \quad .. \quad 11\,a$$

$$B = \frac{Y_1}{Y_2} \quad .. \quad .. \quad 11\,b$$

$$C = A + K_2 \quad .. \quad .. \quad 11\,c$$

$$D = \frac{\mu_{max_1} \mu_{max_2}}{Y_1} X_o \quad .. \quad .. \quad 11\,d$$

$$E = \mu_{max_1} \mu_{max_2} \quad .. \quad .. \quad 11\,e$$

$$F = D + E S_1O \quad .. \quad .. \quad 11\,f$$

$$N = \frac{C K_s}{A F} \quad .. \quad .. \quad 11\,g$$

and L and M can be evaluated from the following relationships:

$$(C + B K_1) = L F + M A + N (BF - AE) \quad .. \quad 11\,h$$

$$B = - E L + M B - N B E \quad .. \quad 11\,i$$

Relationship between S_2 and S_1 may be written as

$$S_2 = S_2O - \frac{Y_1}{Y_2} (S_1O - S_1) \quad .. \quad .. \quad 11\,j$$

Also the equation for peptone removal can be written as

$$t = - \frac{M}{B} \ln \frac{A+B S_2}{A+B S_2O} + \frac{L}{E} \ln \frac{F-E S_2}{F-E S_2} \quad .. \quad 12$$

where S_2O and S_2 is the peptone concentration initially and at any time t. The values of constants A,B,D,E,F,L,M and N are as expressed above and can be obtain by substituting S_1O for S_2O and S_2O for S_1O in the equations 11 a to 11 f.

In order to facilitate the evaluation of constants in the equation 10 two seperate cases may be considered i.e. when electron donor is in excess and when the electron acceptor is in excess.

$$\frac{dx}{dt} = \mu_{max_1} \quad \mu_{max_2} \quad \frac{S_1}{K_{s_1}+S_1} \qquad .. \quad .. \quad 13$$

and

$$\frac{dx}{dt} = \mu_{max_1} \quad \mu_{max_2} \quad \frac{S_2}{K_{s_2}+S_2} \qquad .. \quad .. \quad 14$$

Each of these equations involve two constants μ_{max_1} μ_{max_2} and K_{s_1} or K_{s_2} . These can be evaluated for a batch culture by the graphical analysis method given by Gates and Marler (48) and summarized below.

Equation 7, for a single substrate, on integration yields

$$\ln X_t = \mu_{max}\, t - \left(\frac{YK_s}{X_o+YS_o}\right) \ln \left(\frac{X_t}{X_o}\right) \frac{YS_o}{YS_o+X_o-X_t} + \ln X_t \qquad .. \quad .. \quad 15$$

where X_o and X_t are micro-organisms concentration initially and after time t.

Rearrenging equation 15

$$\frac{1}{t} \ln\left(\frac{X_t}{X_o}\right) = \frac{\mu_{max}}{1+\frac{YK_s}{X_o+YS_o}} - \frac{1}{t} \quad \frac{\frac{YK_s}{X_o+YS_o}}{1+\frac{YK_s}{X_o+YS_o}} \ln\left(\frac{YS_o}{YS_o+X_o-X_t}\right) \qquad .. \quad .. \quad 16$$

Substituting,

$$b = \frac{1}{YS_o} \qquad .. \quad .. \quad 16\,a$$

$$h = X_t - X_o \qquad .. \quad .. \quad 16\,b$$

$$m \quad \frac{\mu_{max}}{1+\frac{YK_s}{X_o+YS_o}} \qquad .. \quad .. \quad 16\,c$$

$$n = \frac{YK_s}{X_o+YS_o} \quad \frac{1+YK_s}{X_o+YS_o} \qquad .. \quad .. \quad 16\,d$$

into equation 15

$$\frac{1}{t} \ln \left(\frac{X_t}{X_o}\right) = n \frac{\ln (1-bh)}{t} + m \qquad .. \quad .. \quad 17$$

The above equation is of linear form if $\frac{1}{t} \ln\left(\frac{X_t}{X_o}\right)$ is plotted against $\frac{\ln (1-bh)}{t}$. To obtain the value of b , a trial and error procedure was adopted. The trial and error procedure is as follow:

(a) Measure X_{o1} S_{o1} X and t for the batch study.

(b) Compute the values of h and $\frac{1}{t} \ln \left(\frac{X_t}{X_o}\right)$.

(c) Estimate a value for h.

(d) Compute the values of $\frac{\ln (1-bh)}{t}$.

(e) Using the arithmatic graph paper plot $\frac{1}{t} \ln \left(\frac{X_t}{X_o}\right)$ 1 Yaxis as a function of $\frac{\ln(1-bh)}{t}$, X axis .

(f) Repeat step c, d, e till a straight line with a positive slope is obtained.

The following expressions for max and K_s are obtained from 16 c and 16 d

$$\mu_{max} = \frac{m}{1-n} \qquad .. \quad .. \quad 15$$

$$K_s = \frac{n}{1-n} \quad \frac{X_o + YS_o}{Y} \qquad .. \quad .. \quad 16$$

Thus obtaining the value of b which linearizes the data and the associated values of intercept m and the slope n, the values of max and K_s can be evaluated.

4.1.3 Design of Experiment

Like all other chemo-organotroph denitrifiers require an organic source of energy and carbon for building up protoplasm and an electron acceptor. In a denitrification system whereas organic matter serve as a source of energy and carbon, the nitrate or nitrite serves as an electron acceptor. Before detailed work was organised some preliminary studies were carried out to assist a design of experiments. Experimental results are given in section 5.1. However, for convinience the findings are summarized below.

Studies made with four exogenous carbon sources that of rawsewage, glucose, acetic acid and peptone to ascertain their suitability as organic substrate, showed that with peptone rate of denitrification was maximum.

Experiment conducted with nitrate and nitrite both being used as electron acceptors, showed that removal of nitrate and nitrite took place simultaneously.

In all the experiments using nitrate as a sole source of electron acceptor, nitrite presence was observed. But the concentration of nitrite was very less at any time being in the range 0-10 mg/l of NO_2-N.

Nitrite is being well established as an intermediate in all the denitrification section. Above findings therefore show that denitrifier accept nitrate as well as nitrite both without reservation.

Removal of high concentration of nitrite or nitrate requires a high concentration of hydrogen donating substrate

to denitrify completely. Raw waste normally not very rich in BOD to meet this demand which neccesitates the use of exogenous source of hydrogen donating substrate. Maximum rate of denitrification being ascertain with peptone, peptone has been choosen as the organic source and has been used throughout the study.

Majumdar (49) in his study with nitrification reported that effluent from a nitrification tank contains larger percentage of nitrite than nitrate. This observation together with earlier described finding that denitrifier do not differentiate between nitrite and nitrate allow the use of nitrite alone as a source of electron acceptor.

Besides these inorganic nutrients were supplementel by adding stock solution of salts resulting in concentration shown in table 4 and tap water which was used to suspend the bacterial mass.

TABLE 4

INORGANIC MEDIUM ADDITIVES

Salts	quantity in gms/l
$MgSo_4$ $7H_2O$	0.2
$FeSo_4$ $7H_2O$	0.05
$Cacl_2$	0.02
$Mncl_2$ $4H_2O$	0.002
Phosphate Buffer, pH 7	0.01M

4.2 Laboratory Set-up

The fill and draw system were employed to determine growth yield and endogenous respiration rate of denitrifiers. Different concentration in four system of micro-organism were obtained by operating these at 10, 25, 50 and 75% wasting.

Completly mixed batch system has been studied to get more information on growth yield and endogenous respiration rate and also to determine the reaction kineties.

4.2.1 Description of Experiment

The denitrification seed was developed from raw sewage by supplying excess of nitrate.

The fill and draw system were employed to determine the growth yield and endogenous respiration rate of denitrifiers. Different concentration of micro-organisms in four systems were obtained by operating these at 10, 25, 50 and 75 percent wasting. The daily schedule of feeding and wasting was as follows.

The fill and draw systems were started by adding seed, NO_2-N 700 mg/l and peptone approximately 2400 mg/l as COD and made up to 2 litre volume. After 24 hours the sides of the bottles were cleaned of solids and make up water added to compensate for evaporation if any. After mixing them thoroughly volume of mixed liquor were wasted from different bottles to obtain desired wasting. The substrates were than added and units were refilled to the initial 2 litre volume with tap water. A certain amount of the mixed liquor wasted was centrifuged at 8000 rpm for 10 minutes to seperate microbial solids from the mixed liquor. The superantant was than analysed for NO_2-N, pH and chemical oxygen demand daily. The mixed liquor

suspended solids were determined by drying and weighing the centrifuged solids.

Completely mixed batch studies were made to determine the reaction kinetics. Few of these runs were made to supplement information on growth yield and endogenous respiration rate.

A column was employed for completely mixed batch studies (details shown in figure 4). Mixing was obtained by recirculating the mixed liquor with a pump. Simples were drawn from centre outlet. Samples were analyzed as described earlier in fill and draw studies.

4.3 Analytical Techniques

4.3.1 Nitrogen Determination

Sulfanilic acid-napthylamine hydrochloride method and Brucine sulfanilic acid method for determination of nitrite-nitrogen and nitrate-nitrogen respectively was used as described in Standard Methods (50). Absorption measurements of color were made by means of Spectronic '20'[1]. The standard calibration curves are shown in figures 5 and 6.

4.3.2 Chemical Oxygen Demand

Dichromate-reflux method as described in Standard Methods (50) was used to determine the chemical oxygen demand (COD) of the samples using 10 ml of 1 N dichromate.

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1 Bousch and Lamb Incorporated Rochester, Newyork, 14602.

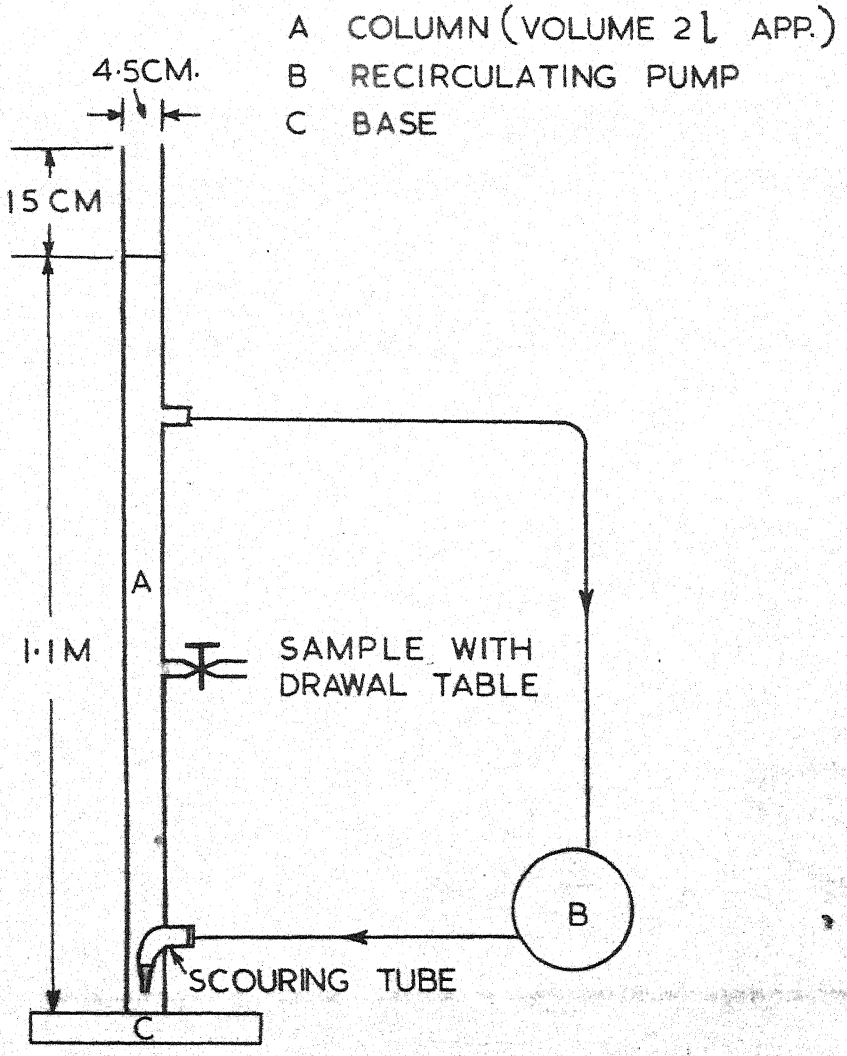

FIG. 4. COLUMN USED FOR COMPLETELY MIXED BATCH STUDIES

NOTE:- NOT TO SCALE

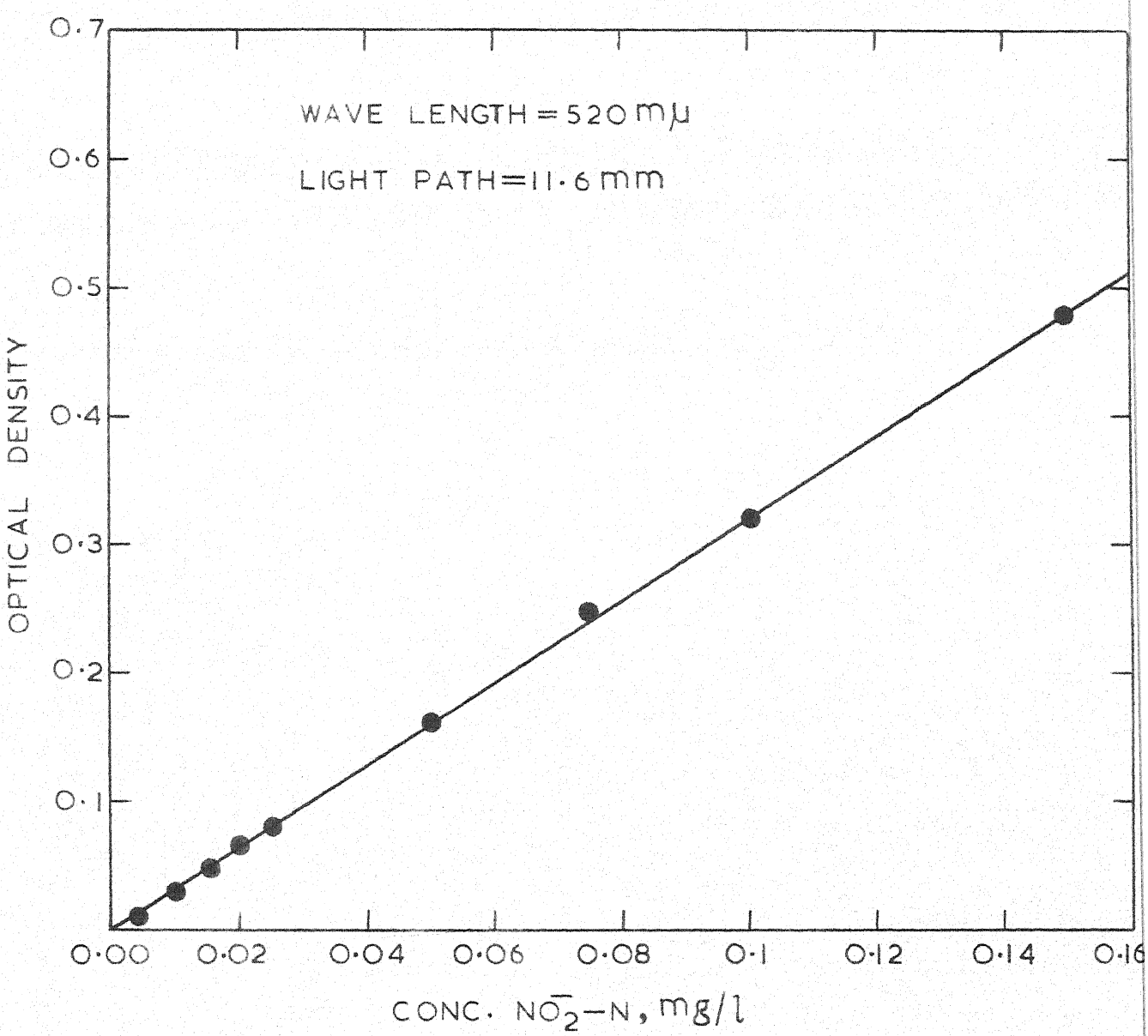

FIG. 5. STANDARD CALIBRATION CURVE FOR NO_2^-–N.

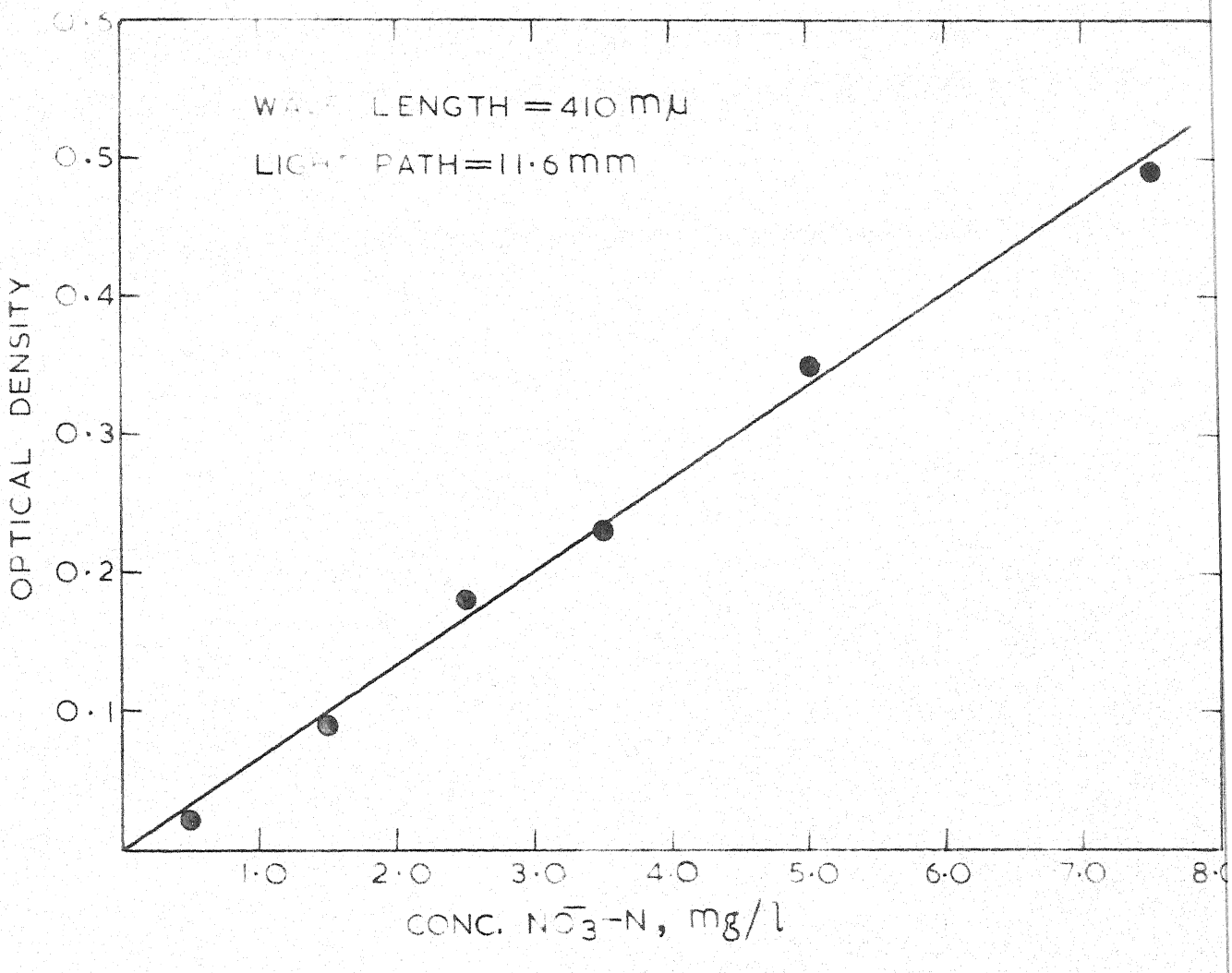

FIG. 6. STANDARD CALIBRATION CURVE FOR NO_3^--N

4.3.3 Suspended Solids

Syspended solids determination was done by drying centrifuged solids at 100°C to constant weight. The time required for drying was determined by weighing a sample of suspended solids after drying it for different times. It was found that after 24 hours there was no appreciable change in weight.

4.3.4 Hydrogen Ion Concentration

pH of the samples was determined with the help of Bechman Expandomatic pH meter[2].

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2 Bechman Instruments Incorporation, Scientific and Process Instrument Division, Fullertone, California 92634.

5. RESULTS AND DISCUSSIONS

5.1 Preliminary Studies

To denitrify a high concentration of nitrogen (700 mg/l or above) it is essential that incoming biochemical oxygen demand in the reactor should also be high in order to provide necessary electrons for denitrification. Usually raw waste does not have high BOD to meet this demand of electrons. It is, therefore, necessary to have a high BOD organic source other than sewage. Studies were conducted to find a suitable electron donor. Experiments were conducted with exogenous carbon sources that of raw sewage, acetic acid, glucose and peptone. The denitrification was established in all these reactors. Denitrification rate observed was maximum with peptone hence it was chosen as the electron donor substrate.

The present study, as mentioned earlier, was undertaken to find out the kinetics of denitrification to complement a nitrification process so as to form a complete method of treatment of fertilizer industry.

Majumdar (49) carried out a study on nitrification of fertilizer industry waste. He reported that effluent from nitrification tank contain 60 to 70% of nitrite-nitrogen and only 10 to 20% of nitrate-nitrogen of total incoming ammonia-nitrogen.

Since a larger percentage of nitrite than nitrate appear in the effluent from a nitrification tank (49), nitrite

only has been taken as a source of electron acceptors in the present study. Study conducted with nitrate and nitrite, both being used as electron acceptors, showed that removal of nitrate and nitrite took place simultaneously (figure 7). Experiment were carried out with nitrate as a sole source of electron acceptor. When denitrification was established, the presence of nitrate was also observed. At any time the concentration of nitrite was very less, being in the range 0 - 10 mg/l of NO_2 N. Furthermore in all the denitrification reactions described in section 2.4.1 nitrite is established as an intermediate. Above observations, therefore, show that denitrifiers do not differentiate between the nitrate and nitrite and accept both without any reservation. Hence, the use of nitrite alone as a source of nitrite will not effect the results.

The ratio of nitrate or nitrite oxygen applied to chemical oxygen demand applied is of decisive influence on the removal of nitrogen. The greater the excess of chemical oxygen demand the greater the chances for complete denitrification of nitrate or nitrite.

The experiments were conducted with different ratio of nitrate oxygen applied to chemical oxygen demand applied. Figure 8 shows the nitrogen removal with varying ratios. The ratios taken into consideration were 2.0, 0.96 and 0.686 and were obtained by varying peptone concentration keeping nitrate nitrogen concentration constant at 700 mg/l.

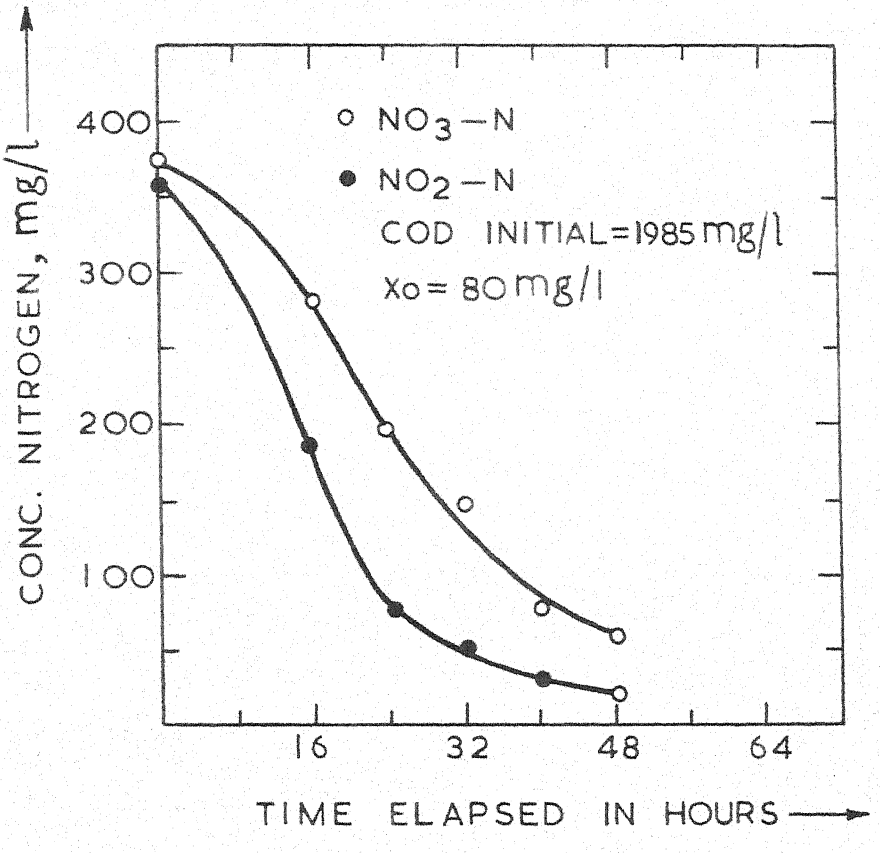
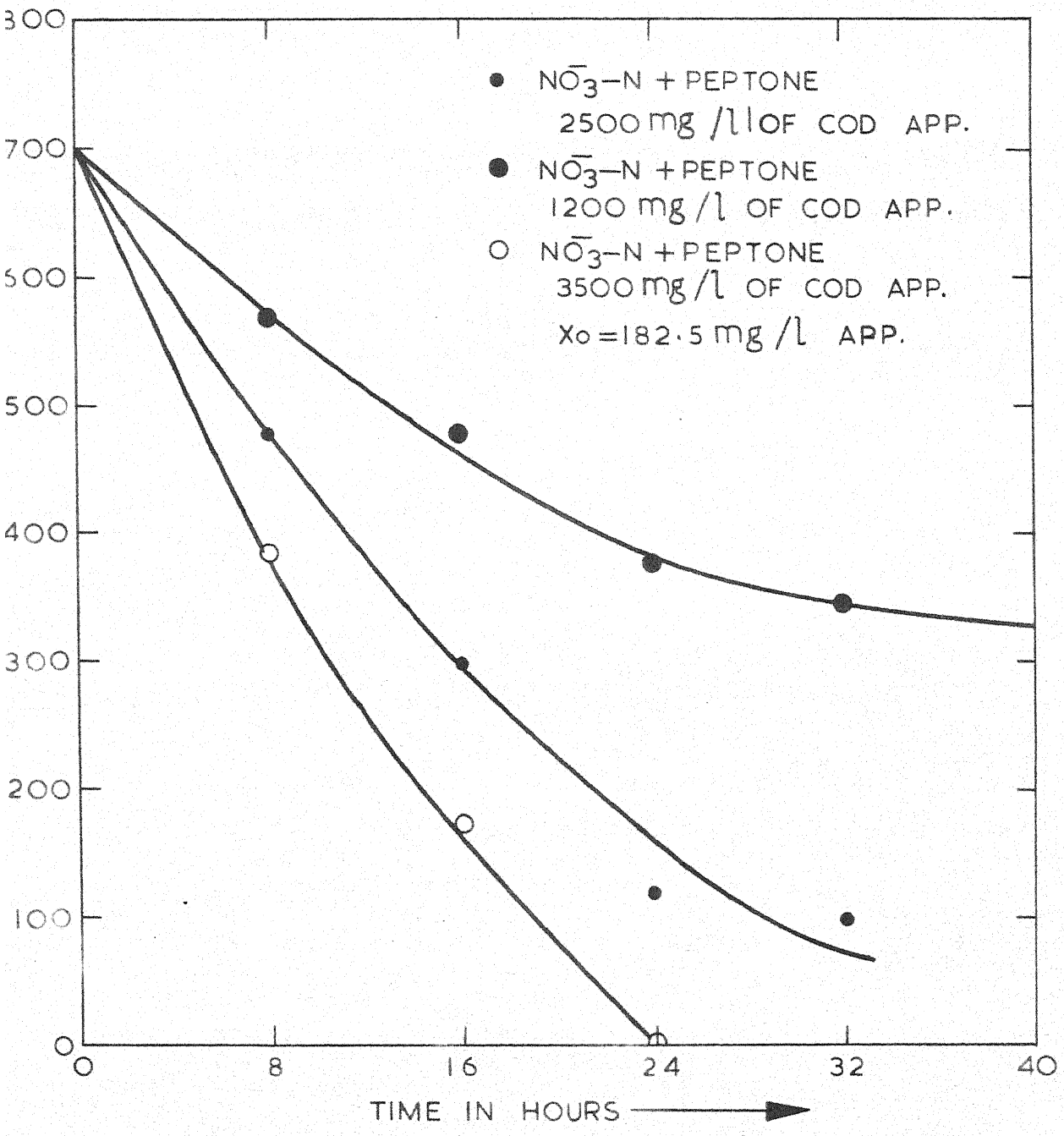


FIG. 7. DENITRIFICATION CURVES



g. 8. DENITRIFICATION CURVES AT DIFFERENT PEPTONE CONC.

It is seen from the figure that the ratio (feed nitrate oxygen to COD applied) approximately .686 resulted in complete denitrification in 24 hours. Increase in the ratio resulted in incomplete denitrification.

Jhonson and Schroepfer (11) in their study of denitrification reported that the ratio of incoming oxygen resources to 5 day BOD in the raw waste of approximately 0.8 results in complete denitrification of the waste. He also observed that an increase in the ratio results in incomplete denitrification. Haltrich (53) in his study reported this limit to be between .6 to .7.

The ratio obtained in the present study in terms of nitrate oxygen to BOD applied rather than COD applied will be slightly higher than .686.

5.2 Growth Yield and Endogenous Respiration Rate Constants

Four fill and draw systems were operated at 10%, 25%, 50% and 75% of wasting. The different percent wastings were adopted to have different concentrations of micro-organisms in the four systems. The observations taken are shown in figures 9 to 12.

It appears from the figures that except 10% waste system all other have attained steady state condition. The increase in the suspended solids is still continued in the 10% waste system. This would have also reached the

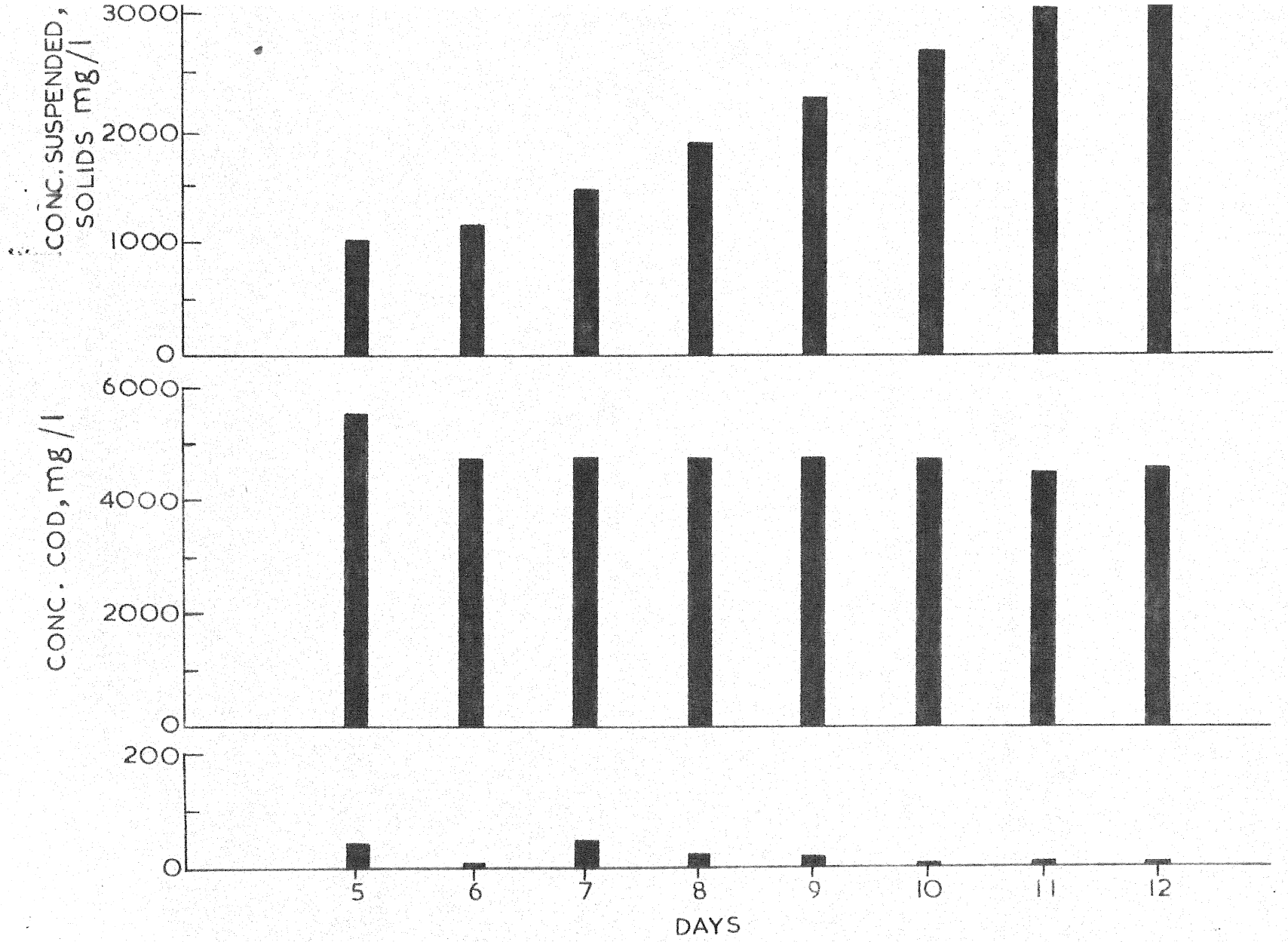


FIG. 9. VALUES OF SYSTEM PARAMETERS AT THE END OF FEED CYCLE, 10% WASTE

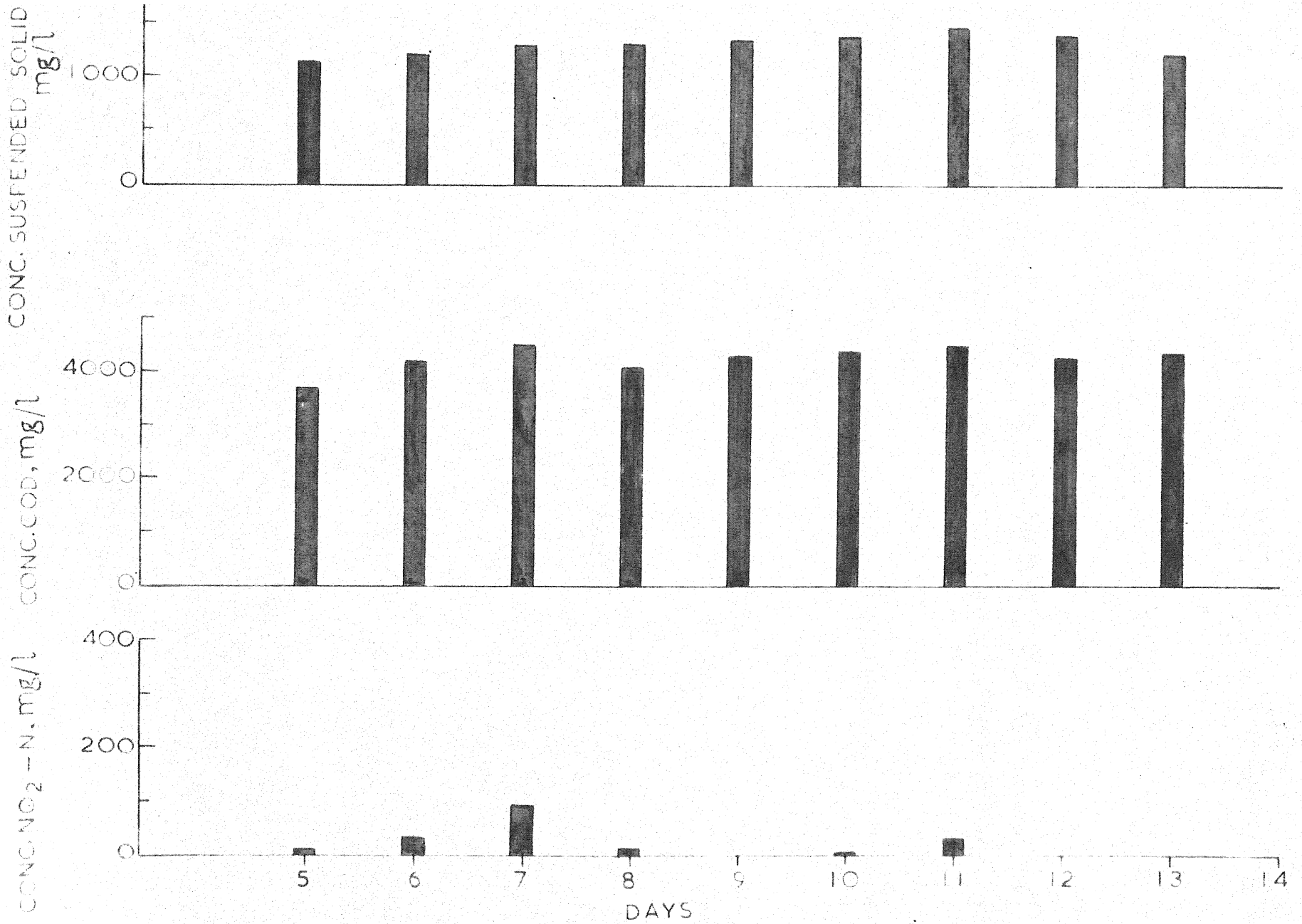


FIG 10. VALUES OF SYSTEMS PARAMETERS AT THE END OF A FEED CYCLE 25% WASTE

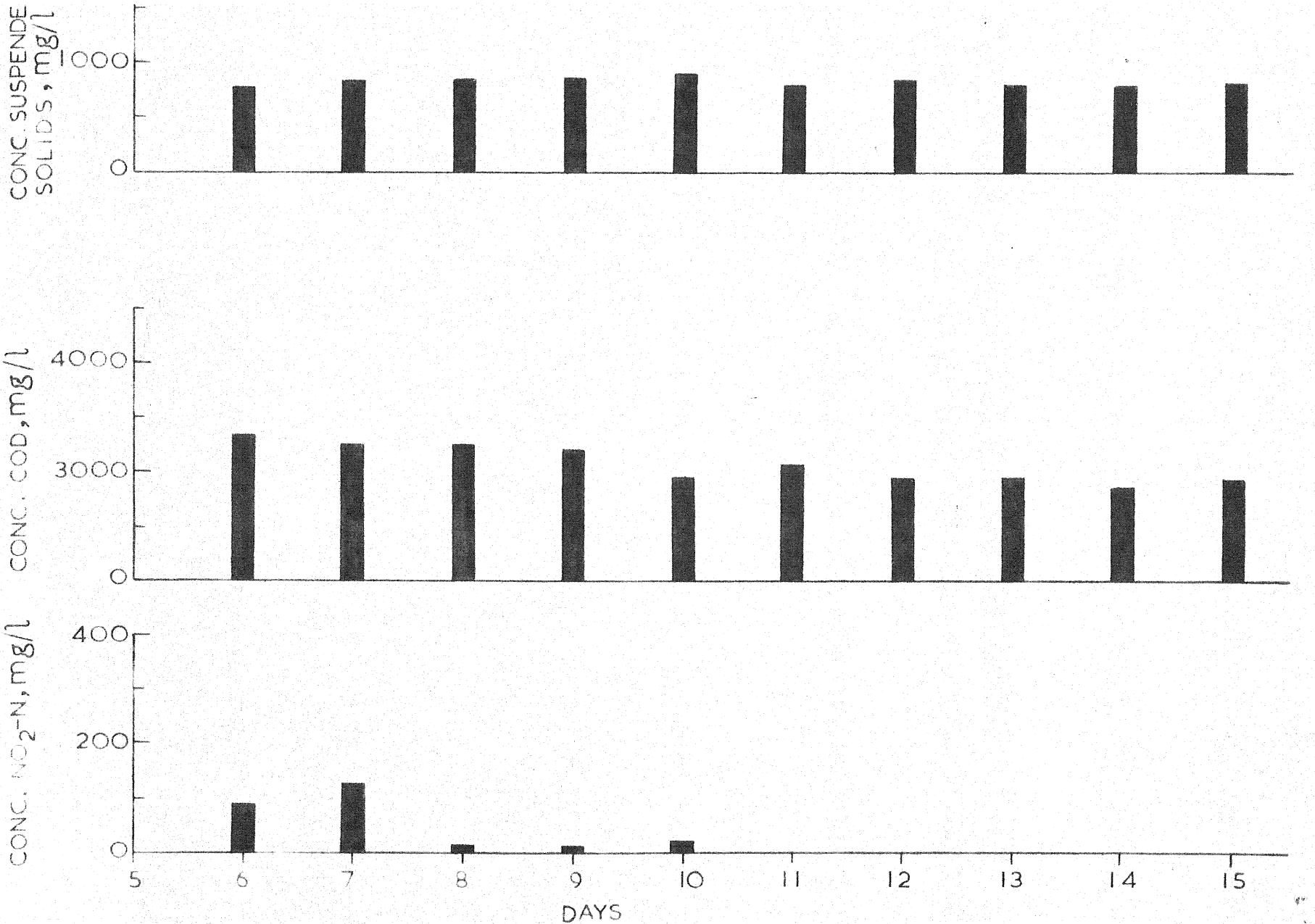


FIG. 11. VALUES OF THE SYSTEM. PARAMETERS AT THE END OF A FEED CYCLE, 50% WASTE

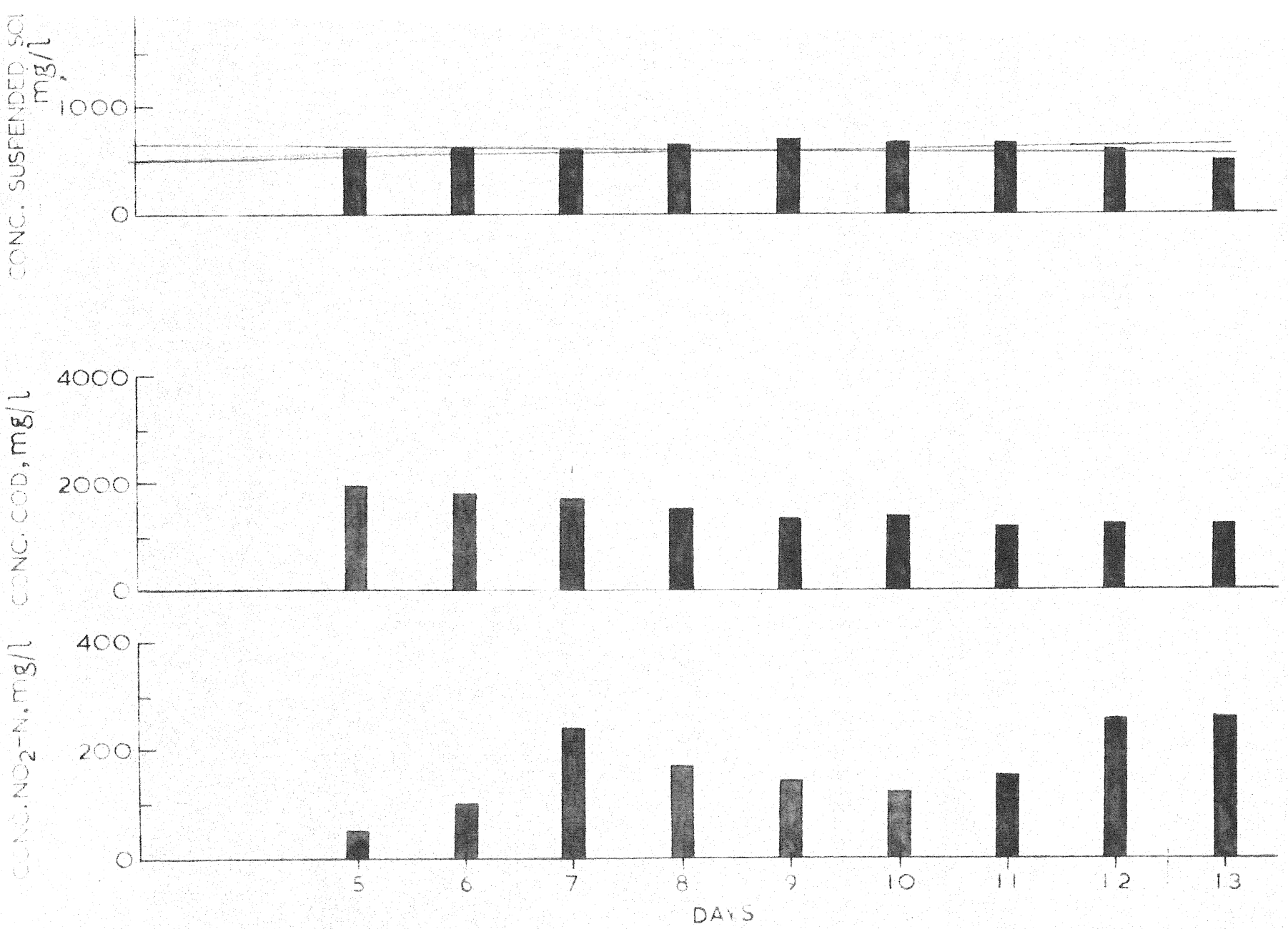


FIG. 1. VALUES OF THE SYSTEM PARAMETERS AT THE END OF A FEED CYCLE, 75% WASTE

equilibrium condition had it been continued for few more days. At the end of feed cycle the steady state values of suspended solids in the 25%, 50% and 75% wasting system may be taken as approximately 1350, 800 and 500 mg/l respectively. On last day of operation suspended solids concentration in the 10% waste system attained a value of 3850 mg/l.

The ratio of nitrite oxygen to COD applied in the feed was maintained approximately as 0.686 to achieve complete denitrification. 90 to 100% denitrification was observed in the reactor with percent recirculation more than 50%. Whereas approximately 75% denitrification resulted in the reactor with 25% recirculation. The reason for incomplete denitrification may be attributed to the fact that micro-organisms in this reactor are in actively growing state (high food to micro-organism ratio) and therefore they use more of COD to build up the protoplasm rather than to use it for respiration.

The pH values in all the systems remain almost constant between the range 9.8 to 10.1.

Since during one feed cycle the mixed liquor in the various systems was not stirred few runs of completely mixed batch studies were also conducted to supplement the information on growth yield and endogenous respiration constants. The observations are shown in table 5.

TABLE 5

DATA FOR DETERMINATION OF GROWTH YIELD AND ENDOGENOUS RESPIRATION RATE CONSTANTS; COMPLETELY MIXED BATCH STUDIES

EXP NO.	Hours from start of experiment								
	2½			20½			24		
	S_1	S_2	x	S_1	S_2	x	S_1	S_2	x
1	750	2685	460.5	110	1655	750	32.5	8830	727.5
2	725	1820	457.5	160	875	780	105	960	686.5
3	1325	1630	920	657.5	1200	1335	625.0	1310	910
4	1377.5	1470	1010	650	1000	1259	640	805	1225
5	350	*	510	60.7	*	680	40.0	*	715
6	38 7.5	*	666.2	50	*	910.0	35	*	916.9

S_1 = Concentration of nitrite-nitrogen in mg/l

S_2 = Concentration of peptone in mg/l

x = Micro-organism concentration mg/l

* = Not estimated

In order to determine the growth yield constants Y_1 and Y_2 and endogenous respiration constants K_{e1} and Ke_2, for the substrates nitrite and peptone respectively. The data obtained from fill and draw studies is reduced for straight line forms as in equations 5 and 6. Figures 13 and 14 show the plots of the data in straight line forms. Least square method of analysis was adopted to obtain the line of best fit. The values of constants obtained for Y_1, Y_2, K_{e1} and K_{e2} are 0.748 mg/mg N_{o2}-N, 0.346 mg/mg COD and 0.108 day^{-1}, and 0.106 day^{-1} respectively.

The rate of endogenous respiration constants obtained are 0.108 and 0.106 per day for nitrite and peptone substrate respectively. These values are almost equal as expected. Since the same organisms are using nitrite and peptone.

In the aerobic biological system the endogenous respiration rate ranges normally between 5 to 15% of biomass per day (51). In the present system the average value for endogenous respiration rate is 0.107 day^{-1} which in terms of percentage comes to be 10.7% of biomass per day and is quite comparable with the values of aerobic systems.

Yield constant when protenious material is aerobically utilized will be near about 0.45 mg/mg of COD (52). In the present system the yield constant comes out to be 0.346 mg/mg COD which may be considered quite comparable. The above observations therefore show that denitrification system is

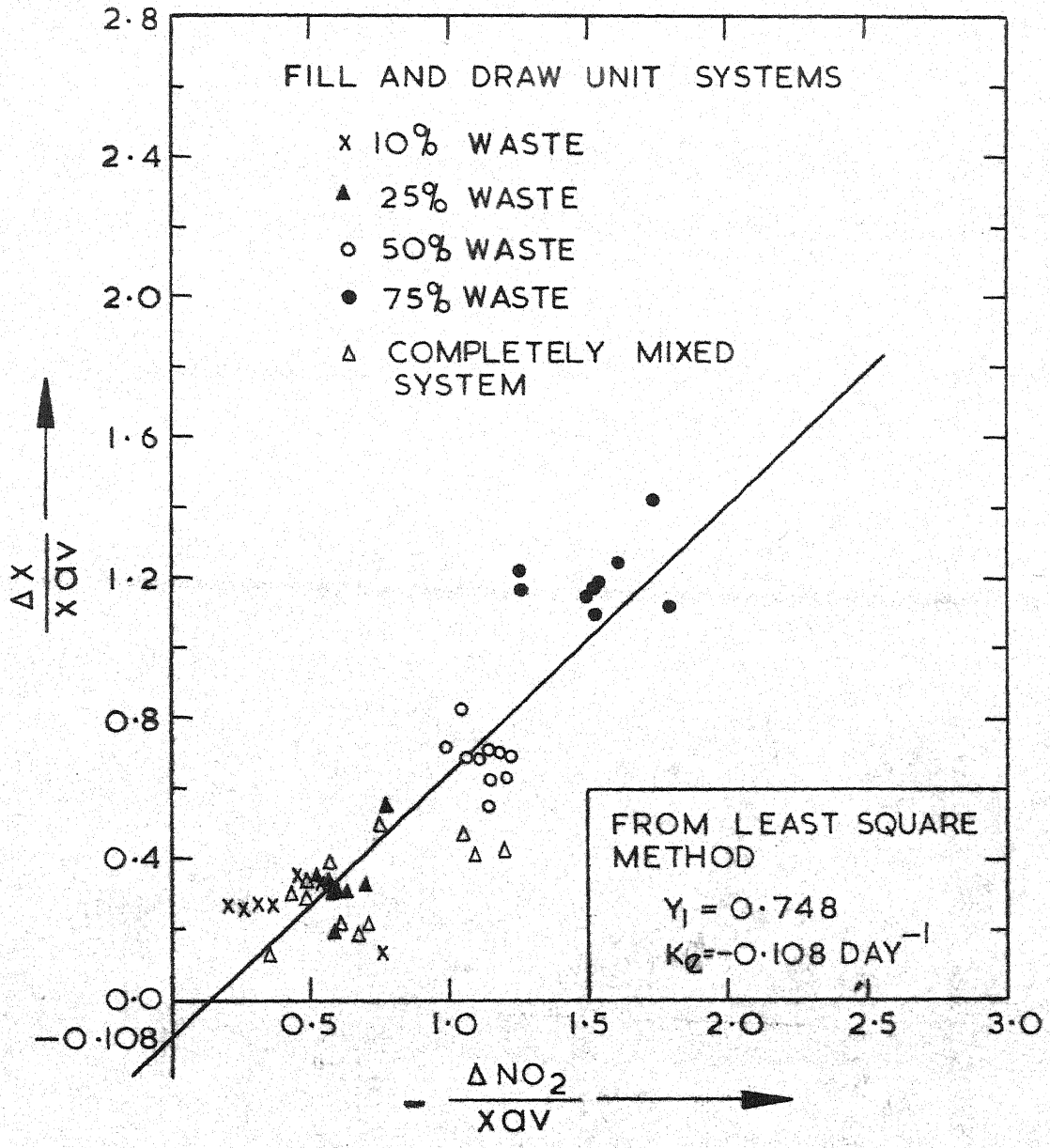


FIG. 13. DETERMINATION OF GROWTH YIELD AND ENDOGENOUS RESPIRATION RATE CONSTANT FOR NITRITE.

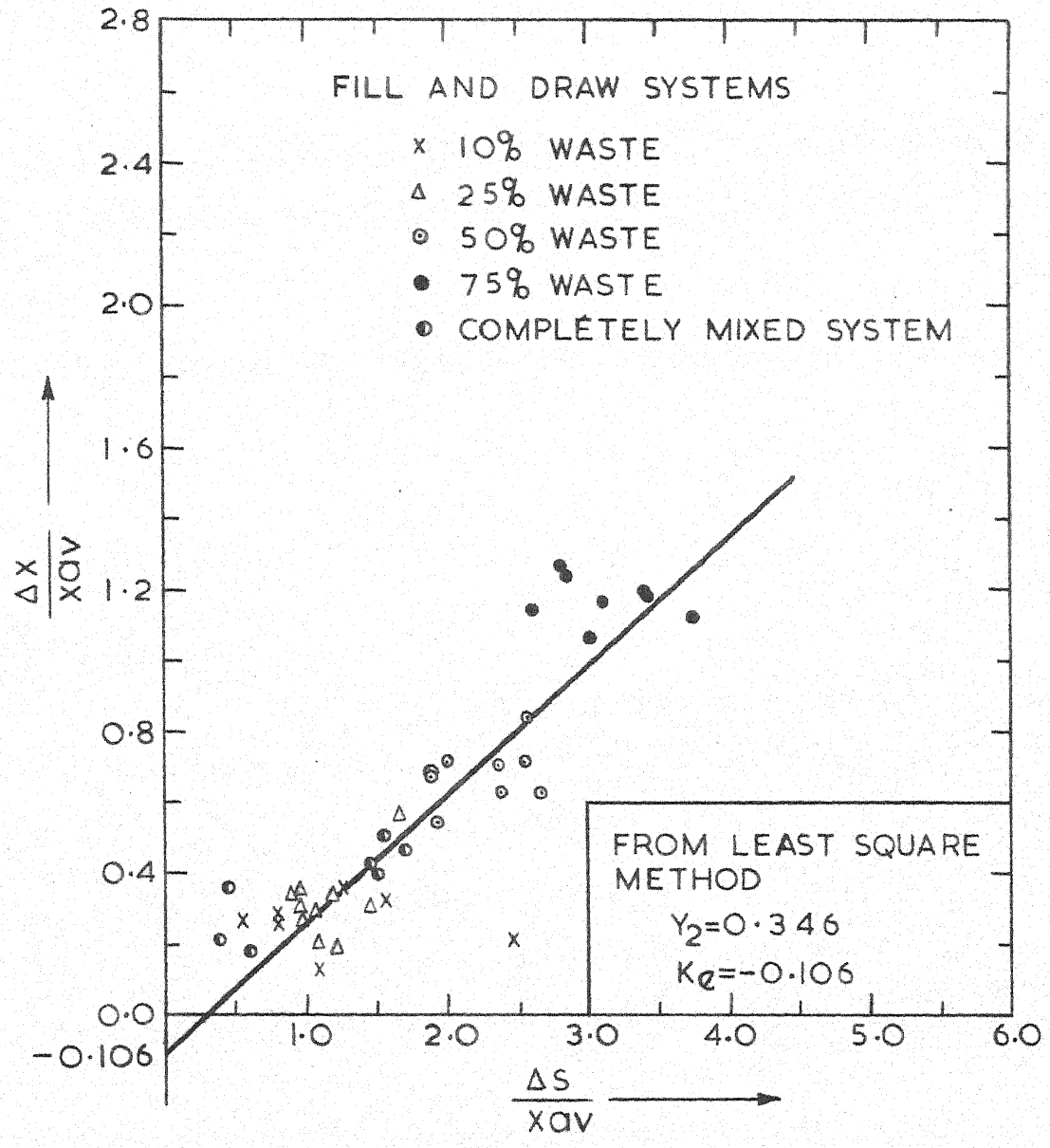


FIG. 14. DETERMINATION OF GROWTH YIELD AND ENDOGENOUS RESPIRATION RATE CONSTANTS FOR PEPTONE

similar to aerobic system in which bound oxygen substitutes oxygen.

If endogenous respiration rate proceeds at high rate (8 to 10% or above) the cell can orcidize their own protoplasm rapidly. Auto-oxidation at the rate of 10% biomass per day is sufficiently high to attempt a total oxidation unit in which endogenous reserve materials may serve as an electron donor untill all nitrate or nitrite ions are reduced. Such a system would conserve an organic source if the synthesized material is utilized for donating electronics.

From synthesis equations a comparison between oxygen requirement for respiration and oxidation of peptone and oxygen released by nitrite can be obtained according to the following example.

Let $S_1 = 800$ mg/l

$x_{av} = 500$ mg/l

Substituting in equation 5 and alopting 0.346 and 0.107 for Y_2 and ke respectively.

Total synthesis = $x + k_e x_{av}$

$= 225 + 54 = 279$ mg/l

assuming COD of suspended solids equal to 1.42 mg/mg (51)

COD of synthesized material = 279 x 1.42 = 410 mg/l

Therefore oxygen consumed in respiration of

COD fed = 800 - 410 = 390 mg/l

Oxygen requirement for sludge

oxidation = 1.42 x.107 x 500

= 76.6 mg/l

Therefore total oxygen requirement

$$= 390 + 76.6 = 466.6 \text{ mg/l}$$

Now for the same increase of micro-organisms nitrate nitrogen consumed will be given by

$$\Delta x = Y_1 \Delta S_1 - k_e \cdot x_{av}$$

or

$$225 = .748 \Delta S_1 - 0.107 \times 500$$

$$S_2 = \frac{279}{0.748} = 373 \text{ mg/l}$$

Many workers have shown that in process of denitrification a significant amount of nitrate or nitrite is lost as N_2O gas. N_2O gas proportion is reported to be between the range 40 to 70% of total amount denitrified depending upon organisms, pH, Temp and other environmental factors (53) (54). On this basis assuming that an equal proportion of N_2 and N_2O gas is formed, the following equation can be written

$$4HNO_2 + 2H = N_2 + N_2O + 4O + 3H_2O$$

i.e. 56 mg of NO_2-N will give 64 mg of oxygen.

Therefore 373 mg/l of NO_2N will give $\frac{373 \times 64}{56}$

$$= 420 \text{ mg/l of oxygen}$$

This value is slightly lower than the computed oxygen requirement. This discrepency could be due to COD of cells being different thann what assumed, the proportion of N_2O formed being higher than N_2, or experimental errors.

5.3 Kinetics of Substrate Removal

Continuously mexed batch reactor studies were conducted to obtain kinetics of denitrification reaction. In order to

evaluate the value of product of growth rate constants, μmax_1 and μmax_2 and saturation constants ks_1 and ks_2 for nitrite and peptone respectively. it is assumed that reaction rate becomes independant of the substrate taken into excess. Keeping the nitrite concentration constant the rate of removal of nitrite will go on increasing with the increase in the peptone concentration and vice versa. Thereafter a limit will reach when further increase will not show an increase in the rate of removal. Experiments were conducted to ascertain the validity of these assumptions and to find the substrate concentrations when the removal rates become independent of one of the substrate.

Experiments were conducted with increasing concentration of peptone, keeping the initial concentration of nitrite constant at approximately 675 mg/l as NO_2-N. The results of experiment are shown in figure 14. The inset shows the initial rate of denitrification at different COD concentrations. It is seen that the limiting concentration of peptone is 4050 mg/l of No_2-N beyond which there is no increase in removal rate with increase in the peptone concentration.

Similar experiments were conducted with increasing concentration of nitrite keeping peptone concentration constant at approximately 620 mg/l of COD. The results of the experiment are shown in Figure 16. The rate of removal of peptone decreased instead of increasing with the increase in nitrite concentration, inset figure 16. Decrease in rate of removal may be because of the poisioning of the system with higher concentration of nitrite. Initial

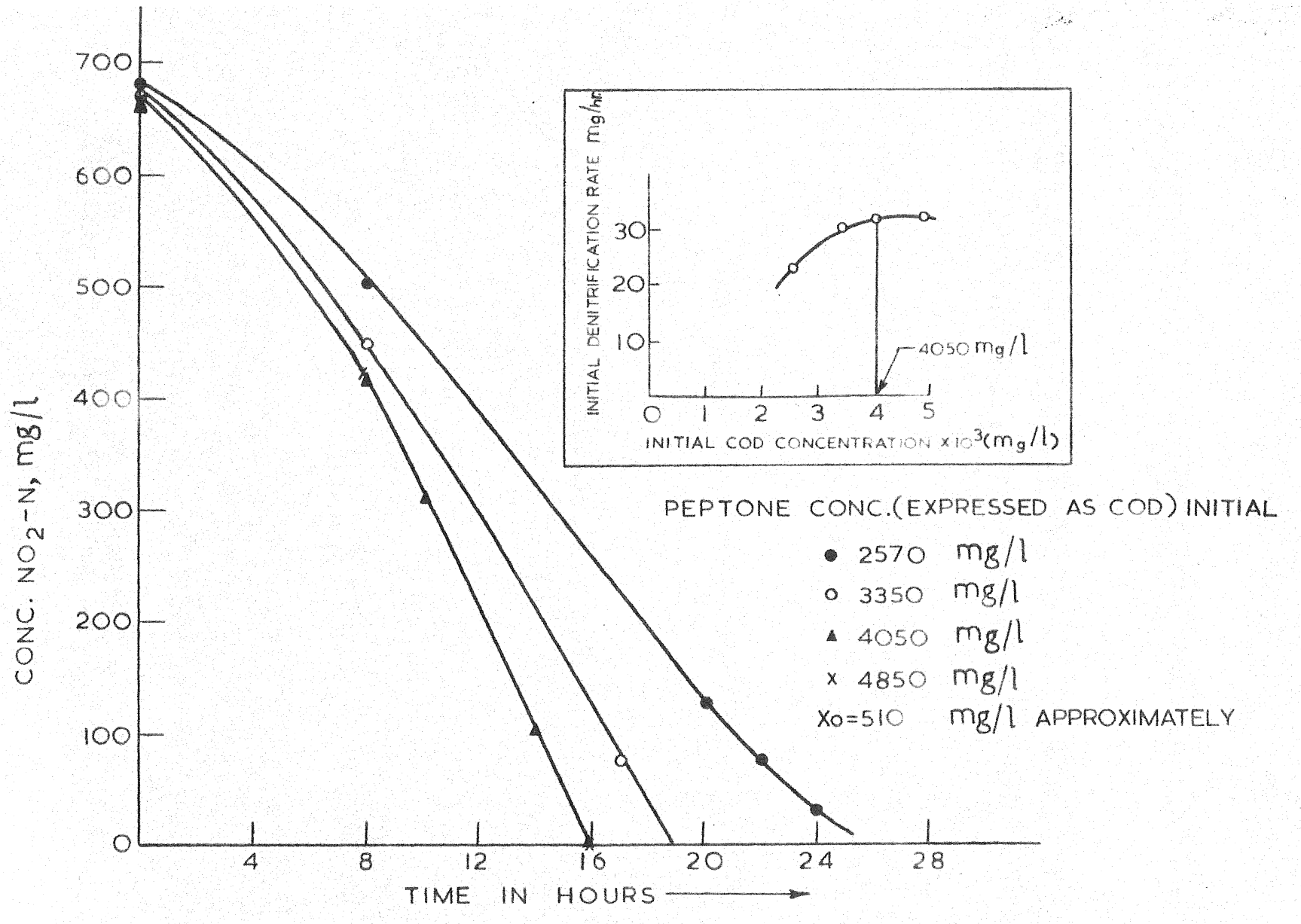


FIG. 15. DENITRIFICATION CURVES AT DIFFERENT PEPTONE CONCENTRATION

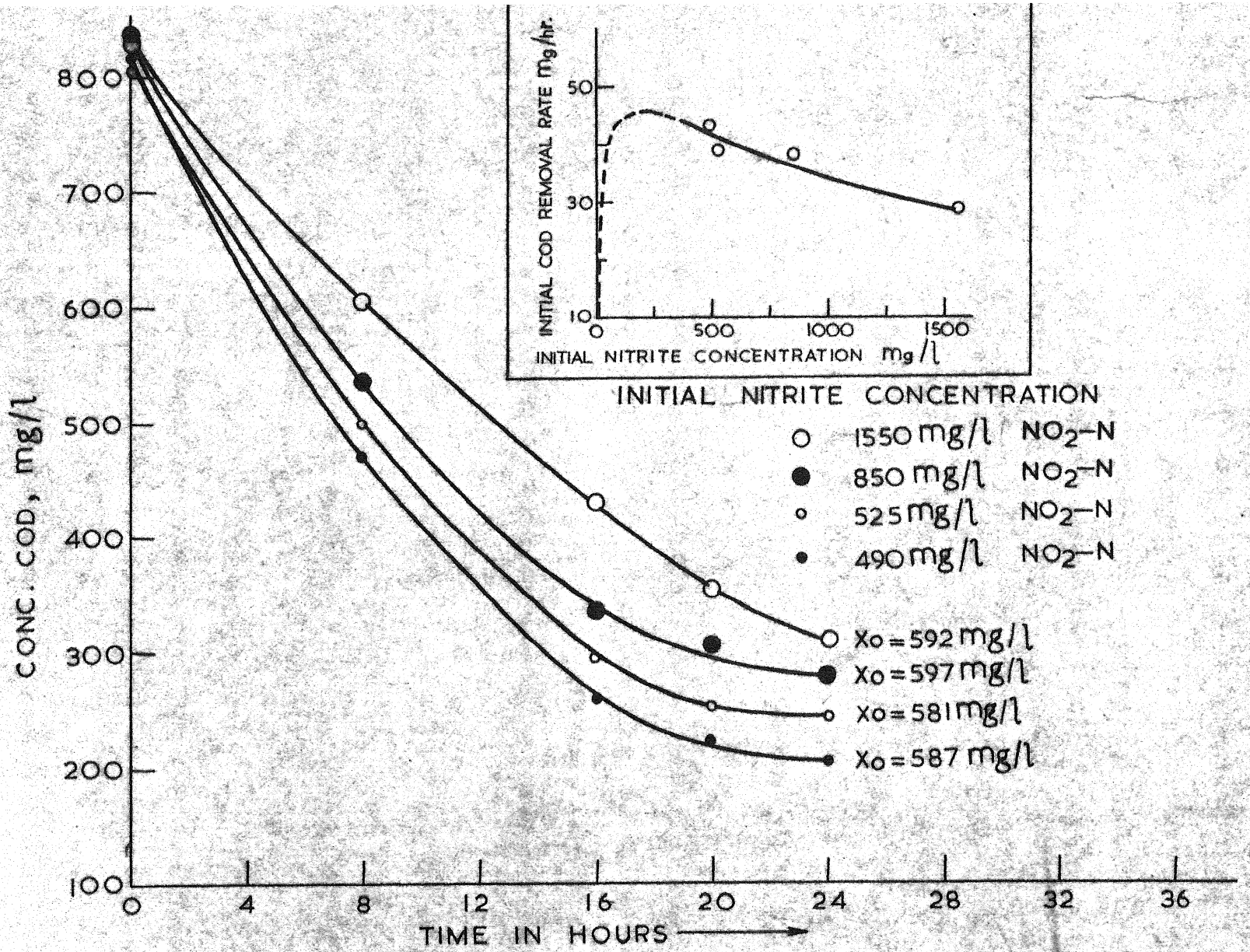


FIG. 16. COD REMOVAL CURVES AT DIFFERENT NITRITE-NITROGEN CONCENTRATION

removal rate of COD in the range 0 - 500 mg/l which may be expected as shown in the inset figure 16 by dotted line. Which indicates that initial COD removal rate goes on increasing with the increase in nitrite concentration achieving a peak value some where in the neighbourhood of 250 - 300 mg/l then it starts decreasing.

The concommitant denitrification for the same experiments is shown in figure 17 and 18. It is seen that amount denitrified in the first 5 hours for all the systems is the same. The COD removal during this period is less when the initial concentration of nitrite is high. This can be explained if it is assumed that due to poisioning of the systems with initially high concentration of NO_2 lesser synthesis of cellular mass is taking place. Denitrification rate for systems having 480 and 500 mg/l COD falls later probably because these system lie in the range when NO_2 concentration limits rate of denitrification, inset figure 16.

A plot made between mg COD consumed/mg NO_2-N consumed and initial nitrite concentration (figure 19) shows that mg COD consumed/mg NO_2-N consumed decreases with higher initial nitrite concentration. The decrease may be because of poisioning of the systems with higher concentration of nitrite.

Above obseervation therefore show that model discussed earlier for single substrate do not apply to the present system in which two substrate are rate limiting as nitrite in high concentration has inhibition effect on the system.

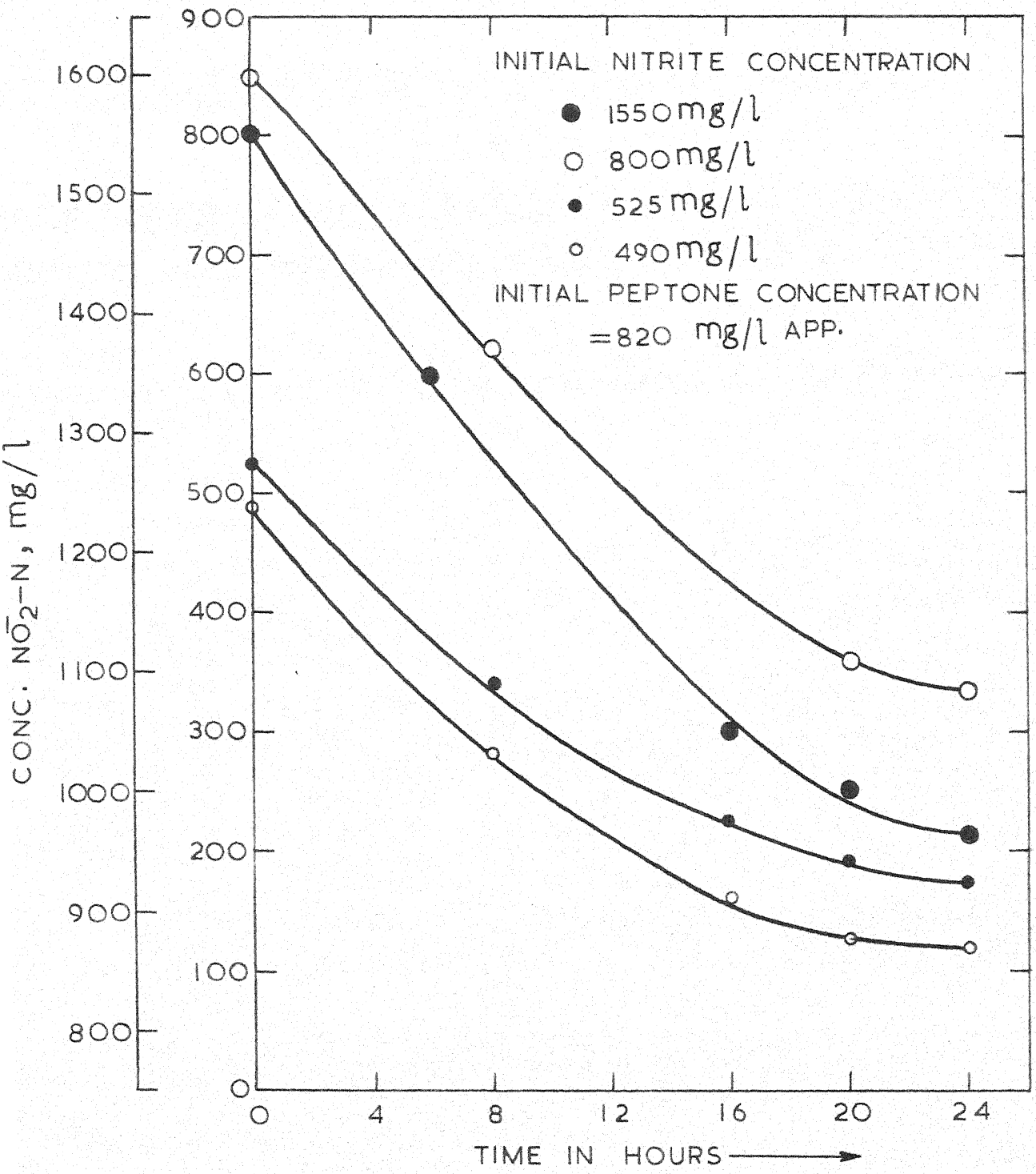


FIG. 17. DENITRIFICATION CURVES AT CONSTANT PEPTONE CONC.

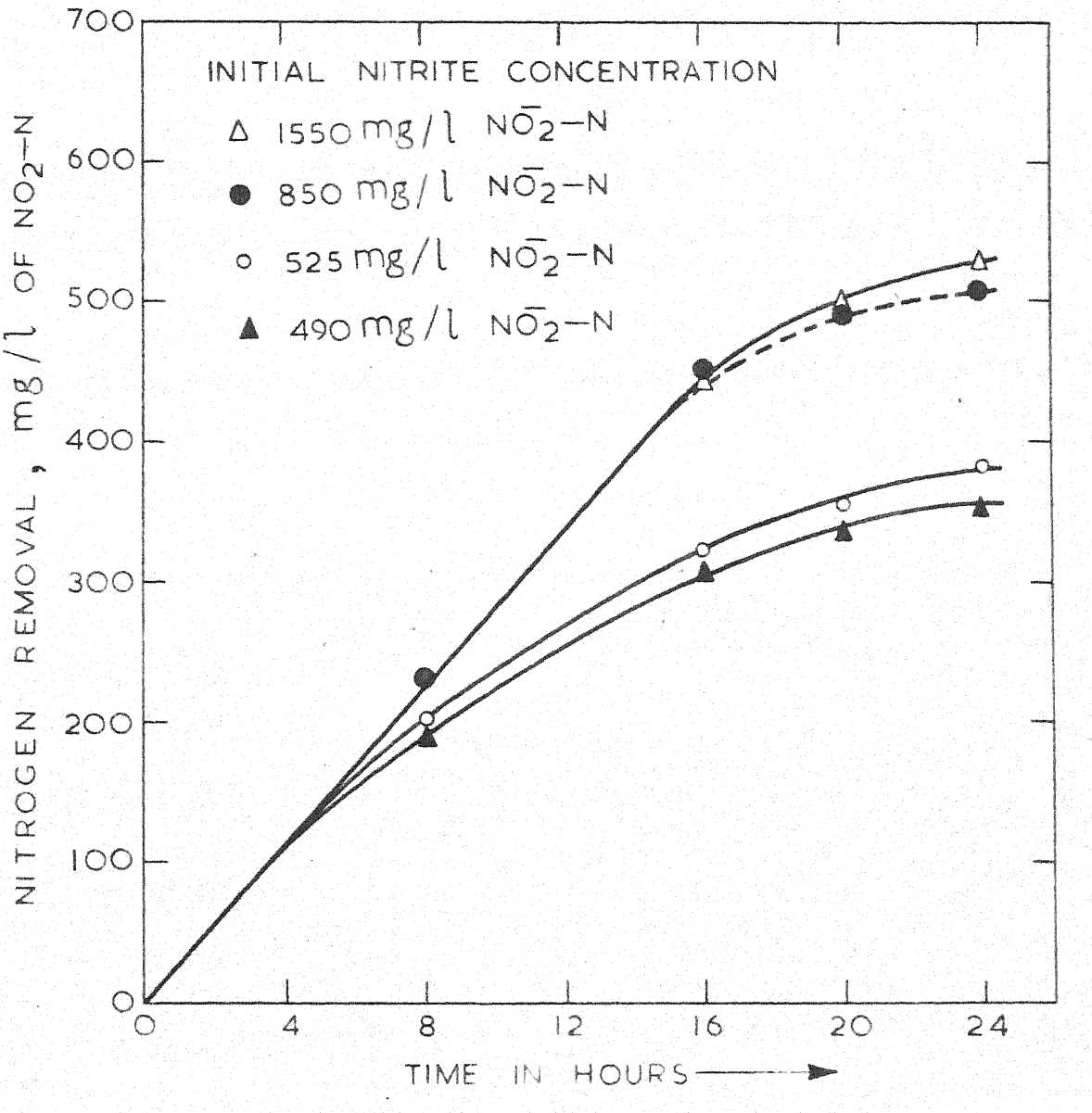


FIG. 18. NITROGEN REMOVAL CURVES AT CONSTANT PEPTONE CONCENTRATION

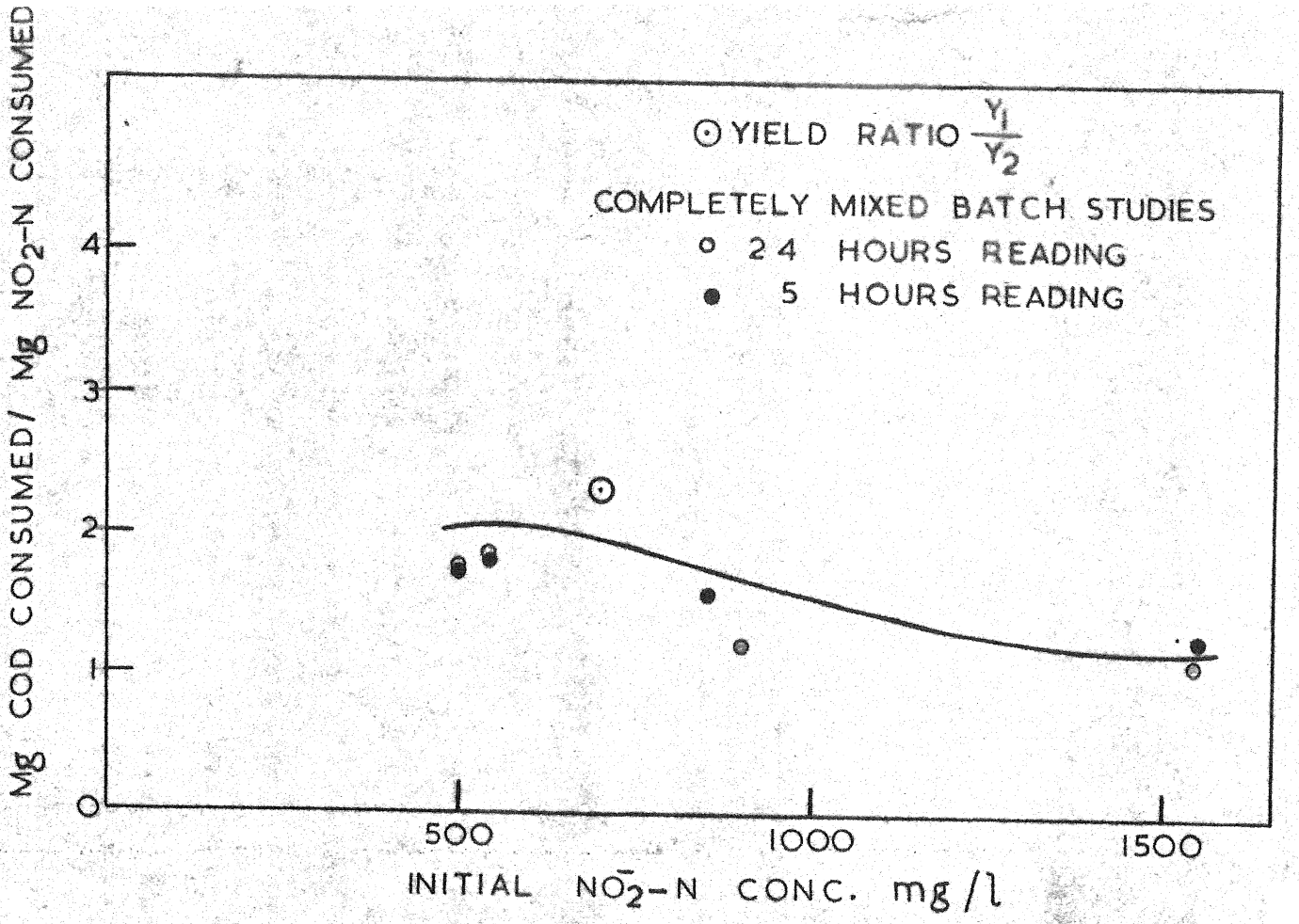


FIG. 19 NITRITE CONCENTRATION EFFECT ON COD REMOVAL

6. CONCLUSIONS

Based on the findings of this study on denitrifications employing fill and draw and continuously mixed batch studies following conclusions may be drawn.

1. Waste enormously rich in No_3 or No_2 concentration may successfully be treated by means of biolgical denitrification treatment to complete denitrification provided sufficient amount of hydrogen donating substrate is present. Thereby treatment of Fertilizer waste consisting high concentration of nitrate and nitrite is possible by means of biological nitrification denitrification.
2. Ratio of incoming oxygen sources to COD applied 0.686 approximately results in complete denitrification.
3. Denitrifiers accept NO_3 nitrate and nitrite both as electron acceptor without differentiation.
4. The values of the yield constants obtained for nitrite and peptone substrate are 0.748 mg/mg No_2-N and 0.346 mg/mg CCD respectively.
5. The value of endogenous respiration constant for denitrification system obtained is 0.107 day^{-1}.
6. The kinetic expression for the removal of single substrate used by Monod (46) does not apply to denitrification system consisting two substrates as one of the substrate (nitrite) in high concentration has inhibitory effect on the system.

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